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POLYCYTHEMIA ASSOCIATED WITH RENAL TUMORS

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Polycythemia of pronounced degree has been encountered in four patients who were found to have hypernephroma. In three the blood abnormality was recognized before the presence of the renal tumor was suspected. In each case a diagnosis of polycythemia vera was considered and in one instance seemed to be supported by the finding of a left upper quadrant mass which was mistakenly identified as an enlarged spleen. The occurrence of gross hematuria eventually led to the discovery of the renal neoplasm in three cases. Collateral evidences of polycythemia vera including leukocytosis, thrombocytosis, and splenomegaly were not present. Neither was there evidence of cardiac or pulmonary disease which might have produced arterial oxygen unsaturation. It seems likely that the relationship between the tumor and the blood disorder was not merely coincidental.

CLINICAL STUDIES

Case 1—JHH # 40 03 65. This 61 year old farmer was admitted to The Johns Hopkins Hospital in 1946 because of a mass in the left side. There was no family history of neoplastic disease or of blood dyscrasia. The patient's previous health had been good and he had had no symptoms of respiratory or cardiovascular disease. About 4 years before admission, he began to lose strength and his weight decreased from 180 to 118 lbs. His family observed that he had developed a more dusky complexion. Two years before admission, he was discovered to have hypertension and at this time complained of dizzy spells, nausea, and vomiting following meals. For about 18 months, the patient had noted a "knot" in the left side of the abdomen which was not tender or painful. He had observed bleeding from his gums on brushing his teeth and had occasional streaks of blood in his urine, though there were no urinary symptoms except nocturia two or three times. Eight months before admission the patient had a severe episode of epistaxis. On another occasion he lost consciousness for about 2 hours following which there was weakness of the mouth for several days. Three weeks before admission there was massive bleeding from the urinary tract.

Physical examination on admission showed the temperature to be 99.2°, pulse 68, respirations 22 and blood pressure 155/100. The patient appeared well developed and poorly nourished. The face, ears and neck showed a striking deep rose hue and the lips were cyanotic. The conjuctivae were very red and the sclerae suffused. The retinal veins appeared dark. Numerous dilated venules were seen over both cheeks. The tongue and mucous membranes of the mouth and throat were deep scarlet in color. Small lymph nodes were palpable in the axillary and inguinal areas. Respiratory excursions were good and the lungs were clear. The heart was not enlarged to percussion and no abnormalities were noted. The peripheral vessels showed considerable tortuosity and sclerosis. The abdomen appeared full and there were prominent veins on the anterior and lateral abdominal wall. There was no tenderness and

no signs of fluid. The liver edge was palpable at the costal margin on inspiration. The upper two-thirds of the left side of the abdomen was filled by a non-tender mass, extending from the costal margin almost to the anterior superior iliac spine. There was no clubbing of the digits and no other significant abnormality.

Blood studies on admission showed the red cell count to be 7.2 million per cmm., hemoglobin 18.6 gm. per 100 ml., hematocrit value 72 per cent and white cell count 5,050 per cmm. Platelets numbered 210,000 per cmm. The differential count was normal. Bone marrow aspirated from the sternum showed no abnormality and 17 per cent of the nucleated cells were erythroid. Urine was clear and straw-colored; the specific gravity was 1.021 and there were no cells in the sediment. The blood non-protein nitrogen ranged between 49 and 60 mg. per 100 ml. A PSP test showed 55 per cent excretion in two hours and urea clearance was 62 per cent of the normal standard. Serum uric acid was 4.3 mg. per 100 ml. The basal metabolic rate was +1. X-ray of the chest showed some enlargement of the left ventricle and dilatation of the aorta but the lungs were clear. An electrocardiogram was normal.

At the time of admission the patient was thought to have polycythemia vera and the mass in the left flank was considered to be an enlarged spleen. Three thousand ml. of blood were removed during a period of one week and the hemoglobin concentration fell from 20 to 14.5 gm., and the hematocrit value from 65 to 49 per cent. Blood pressure decreased to 135/80. Because of the history of hematuria, urographic studies were performed. Intravenous and retrograde pyelograms showed no abnormality of the right kidney but the left kidney seemed to be pushed downward by a large mass. There was marked deformity of the left renal pelvis characteristic of that produced by a renal tumor. Subsequently, a trans-peritoneal nephrectomy was performed. At operation a large vascular tumor of the left kidney was seen extending through the peritoneum in the region of the tail of the pancreas and into the left renal vein. The tumor was invading the spleen which was only slightly enlarged, and it was obvious that it was the tumor and not the spleen which had been palpable. A portion of the spleen, the tail of the pancreas and the left adrenal gland were excised with the tumor mass. Sections of the tumor showed typical features of hypernephroma. Sections of the spleen showed some hyaline tubercles and arteriosclerosis but no other abnormalities. It is noteworthy that at the time of operation and following the removal of 3 liters of blood the patient continued to present a strikingly plethoric appearance. Convalescence following the operation was uneventful and there was no evidence of bleeding. However, the hematocrit value dropped to 28 per cent and the hemoglobin to 9.8 gm. two weeks after the surgical procedure. Attempts to communicate with the patient following his discharge from the hospital have been unsuccessful and his subsequent course is unknown.

Case 2—JHH #72 10 98. This 32 year old white chauffeur was admitted to The Johns Hopkins Hospital in 1955 because of recurrent hematuria for more than one year. The family history was not pertinent and the patient's previous health had been good. About 15 months before admission the patient passed blood-tinged urine and had colicky pain in the flank. Intravenous and retrograde pyelograms performed at that time were reported as normal, but several small stones were seen in the bladder and were thought to be the cause of his symptoms. Five months later there was recurrence of pain and hematuria and again cystoscopy and retrograde pyelograms were reported as normal. Small bits of sand seen in the bladder were considered to be the cause of bleeding. In the three months prior to admission, the patient had episodes of painless hematuria as well as further episodes of colic with passage of blood clots. He passed no stones and had no chills, fever or dysuria.

On admission the temperature was 98.8°, pulse 92, respirations 16 and blood pressure 128/84. The patient appeared to be a robust and athletic young man with a deep tan. The general

physical examination showed no significant abnormalities. There was no cyanosis and no clubbing of the fingers. The lungs were clear. The heart was not enlarged and the sounds were normal. No organs or masses could be felt on abdominal examination, but there was some tenderness in the left costovertebral angle.

Blood studies on admission showed the hematocrit value to be 70.8 per cent, hemoglobin 21.5 gm. per 100 ml. and red cell count 8.64 million per cmm. White cell count was 9,200 per cmm. with normal differential count and the platelets were 280,000 per cmm. Urinalysis showed no abnormality except for the presence of red blood cells. X-ray of the chest showed rather prominent vascular markings in the lungs and some fullness of the right ventricle but no cardiac enlargement or other significant abnormalities. Intravenous and retrograde pyelograms revealed deformities of the collecting system of the left kidney, suggestive of an intra-renal neoplasm. At operation numerous large veins were seen over the surface of the left kidney, which contained a tumor mass in the central portion. The kidney was removed along with several large hilar lymph nodes. Sections of the tumor showed it to be a hypernephroma with pleomorphic cells and numerous mitoses. There was invasion of the veins and lymph nodes. Postoperatively the patient received x-ray therapy to the tumor bed. Convalescence was uneventful. Seven weeks after the operation and after the completion of the x-ray therapy, the hematocrit value was 61.8 per cent, hemoglobin 19.0 gm. and red cell count 7.12 million. About 6 months following the surgical procedure, a large firm mass appeared in the neck. Following local x-ray therapy this mass disappeared. One year after resection of the tumor the patient had developed dyspepsia and discomfort in the left upper quadrant, and had lost 10 lbs. in weight. Nevertheless, he had remained remarkably well. At this time he presented an appearance of extraordinary plethora, with pronounced suffusion of the conjunctivae, fullness of the veins, had a reddish cyanosis of the skin and mucous membranes. The spleen was not palpable and no masses could be felt in the abdomen or in other areas. Examination of the blood showed the red cell count to be 8.2 million per cmm., hematocrit value 70.5 per cent, hemoglobin 20.9 gm. per 100 ml., reticulocytes 0.2 per cent, white cell count 11,600 with normal differential count, and platelets 334,000 per cmm. Serum uric acid was 7.0 mg. per 100 ml. The patient returned 14 months after operation because of severe left-sided abdominal pain. He again was strikingly plethoric in appearance. No masses were palpable in the abdomen. At exploratory laparotomy, large tumor-bearing nodes were seen in the abdomen. At this time the hematocrit value was 64.8 per cent. Detailed studies of respiratory function showed no abnormality and the arterial oxygen saturation was normal.

Case 3—JHH #72 23 94. This 54 year old real estate agent was admitted to The Johns Hopkins Hospital because of painless hematuria of 5 days duration. There was no family history of cancer or of blood disorders. The patient was known to have had mild hypertension for years and had occasional episodes of palpitation and headache. For 2 years he had had mild intermittent epigastric discomfort, tending to come on when he was hungry and to be relieved by eating. There was no weight loss. One year prior to admission the patient was examined by his physician and was found to have polycythemia. Blood studies at this time showed a red cell count of 6.5 million per cmm. and hemoglobin 19.6 gm. per 100 ml. White cell count was 12,000 and the differential count was reported as normal. A diagnosis of polycythemia vera was made and 500 ml. of blood were removed on each of three occasions. In the year prior to his admission he had had frequent mild epistaxes. Five days prior to admission he experienced profuse painless hematuria. The urine cleared but subsequently there was recurrence of gross bleeding.

On admission the temperature was 98.0°, pulse 96, respirations 16, and blood pressure

150/100. The patient was well developed, moderately obese and presented a definitely plethoric appearance. The face and upper thorax were red and there were many small dilated venules. There was no cyanosis and no clubbing of the fingers. Examination of the fundi showed changes of early vascular sclerosis. The heart was not enlarged and there were no murmurs. The lungs were clear. The abdomen was obese and protuberant. The liver and spleen were not palpable and no masses could be felt, but the left kidney seemed to be enlarged. The hematocrit value on admission was 51.2 per cent, hemoglobin 15.4 gm. per 100 ml. and red cell count 6.31 million. White cell count was 10,300 with normal differential count. Platelets numbered 282,000 per cmm. The urine on admission was grossly bloody. Intravenous pyelograms demonstrated an enlarged and non-functioning left kidney. Retrograde pyelograms showed elongation, flattening and blunting of the calyces on the left, changes which were thought to be compatible with tumor. An x-ray of the chest showed no abnormality. Gastrointestinal x-rays showed a duodenal deformity compatible with a chronic peptic ulcer. There was considerable bleeding at nephrectomy and the patient was given 2 units of blood. Sections of the tumor showed a vascular hypernephroma which was invading the veins but which did not extend into the perirenal fat. At the time of discharge from the hospital, the hematocrit value was 44.2 per cent. The patient returned for re-examination four months after the operation. At this time he complained of some generalized weakness but had no other complaints. There had been no recurrence of epistaxis and the plethoric appearance which had previously been present was no longer apparent. Blood pressure was 150/90. At this time the hematocrit value was 44.3 per cent, white cell count 12,000 with normal differential count and platelets 289,000. On examination one year after nephrectomy the patient appeared to be well. There were no evidences of recurrence of the tumor and no signs of polycythemia. The spleen was not palpable. Hematocrit value was 49 per cent. Fourteen months after operation the patient returned because of intermittent severe headaches. At this time he presented a decidedly plethoric appearance. The liver edge was felt 3 finger-breadths below the costal margin but the spleen was not palpable. No masses were felt in the abdomen. Red cell count was 6.00 million per cmm., hemoglobin 16.5 gm. and hematocrit value 51.0 per cent. White cell count was 6,900 and platelet count 204,000. Serum uric acid was 8.0 and 7.1 on two determinations. He was treated by removal of 1,000 ml. of blood.

Case 4—CHH #94378. This 46 year old driver of a beer truck was admitted to the Church Home and Hospital in 1956 because of severe headache of two days duration. He had been known to have hypertension for 2 years, with blood pressure about 190/110. For 6 or 8 months he had had frontal and occipital headaches of severe degree. He had been observed to have a florid complexion which was thought to be the result of the hypertension and the ingestion of alcohol. For 2 or 3 years he noted that he bruised easily. At the time of admission he had had headache for 2 days, associated with nausea and vomiting.

On admission he was afebrile and the blood pressure was 186/110. He appeared flushed and florid with a slightly cyanotic hue. There was no clubbing of the fingers. The conjunctivae were congested. The retinal vessels showed moderate arteriosclerotic changes. The heart was enlarged to the left but the lungs were clear. The liver and spleen were not palpable and no masses were felt in the abdomen. There were two hematomas on the lower extremities. The neurological examination showed no definite abnormality.

The hematocrit value was 68 per cent, red cell count 7.03 million per cmm. and leukocyte count 13,600 with 92 per cent neutrophils. Bleeding and clotting times were normal and platelets appeared normal on the blood smear. Urine contained a small amount of protein but the sediment contained no cells or casts. The NPN was 72 mg. per 100 ml. Spinal fluid pressure was 300 mm. water, the fluid was xanthochromic and contained red and white cells.

X-ray of the chest showed prominence of the left border of the heart. The lungs were clear except for minimal congestive changes.

Repeated venesections were performed and 3,150 ml. of blood were removed over a period of 18 days. At the end of this period the patient still presented a florid appearance. His general condition improved, there was marked decrease in the blood pressure, and the NPN returned to normal. Hematocrit value on the day following the last phlebotomy was 64 per cent, red cell count 6.31 million per cmm., and white cell count 5,000. The liver and spleen could not be felt. The patient was considered to have polycythemia vera and was given 4 mc. of radioactive phosphorus. One month later the hematocrit value was 54 per cent and white cell count 6,900. In the subsequent month the hematocrit value rose to 59 per cent and 500 ml. of blood were removed. About one week thereafter the patient had sudden severe abdominal pain in the right upper quadrant and right costovertebral angle. There were no urinary symptoms. On examination he was found to have tenderness in the areas in which he had complained of pain and resistance in the right upper quadrant. Urine contained no protein and no cells or casts on repeated examination. Hematocrit value was 56 per cent, white cell count 7,300 and platelet count 170,000. Serum uric acid was 4.6 mg. per 100 ml. PSP excretion was 60 per cent in the first 30 minutes. Intravenous and retrograde pyelograms showed marked enlargement of the upper portion of the right kidney with displacement of the superior calyces. These abnormalities were considered indicative of a tumor involving the kidney. Urinary catacholamines were not increased and the injection of histamine did not cause a rise in blood pressure; thus it appeared that the patient did not have a pheochromocytoma. At operation the upper pole of the right kidney was found to contain a very large mass which extended across the midline and almost surrounded the vena cava. Because of the extent of the tumor and its adherence to surrounding structures, nephrectomy could not be performed. Biopsy of the tumor showed typical features of hypernephroma.

Sections of the renal tumors from each of the four cases were reviewed by Dr. Stanton L. Eversole, Department of Pathology, The Johns Hopkins Medical School. Each showed clear cell carcinoma, typical of hypernephroma. In one (Case 2) the cells appeared larger and more pleomorphic than is usual and some cells contained hyaline droplets. However, there were no morphologic features which distinguished these tumors from those encountered in other patients without polycythemia.

Plasma obtained from one patient (Case 2) was infused into an infant with hydrocephalus in an effort to determine whether an erythropoietic substance might be present. A single intravenous injection of 15 ml. of plasma per kilogram of body weight was not followed by a rise in reticulocyte count or hematocrit value during a period of observation of one week. Further efforts will be made to detect erythropoietic activity in the plasma which was obtained from our patients.

The records of The Johns Hopkins Hospital in cases of proved hypernephroma were surveyed. Hemoglobin or hematocrit values on admission were available in 139 cases. The results of these determinations are shown in Figure 1. The majority of values were in the normal or subnormal range. In 5 cases, values were distinctly high. Hematologic studies were inadequate for further evaluation in 2 cases. The remaining three are those described in this report.

FIG. 1. Initial hemoglobin or hematocrit values in 139 cases of hypernephroma.

DISCUSSION

Polycythemia vera is a disorder which seems most likely to represent a relatively benign neoplastic proliferation of the bone marrow. In this disease there tends to be hyperplasia of all of the marrow elements with resultant erythrocytosis, leukocytosis and thrombocytosis. Sooner or later the spleen usually becomes enlarged, often to a very considerable degree. The uric acid concentration of serum may be elevated and the urinary excretion of uric acid is often increased. Crystals of this substance may form in the urinary tract, at times causing renal colic and hematuria. Rupture of congested vessels or infarction of the kidney may also give rise to hematuria. Vaquez (1) described the occurrence of hematuria in the first reported case of polycythemia vera.

Polycythemia has uncommonly been associated with a variety of tumors. In some instances an unrelated neoplasm appears to have occurred coincidentally with polycythemia vera (2, 3). An occasional coincidence of these diseases is not surprising since polycythemia vera occurs at an age period in which neoplastic disease is most prevalent. The simultaneous occurrence of polycythemia vera and myeloma in several instances has led to the suggestion that these diseases may be related through a neoplastic change in a common stem cell in the bone marrow (4). Polycythemia vera occasionally terminates as acute leukemia, indicating that some relationship exists between these disorders of the hematopoietic system.

In other instances, polycythemia appears to be secondary to the presence of a tumor. The occurrence of polycythemia in patients with neoplasms of the central nervous system has received much attention. Cerebellar hemangioblastoma is the tumor most frequently reported to be associated with polycythemia, although neoplasms of other types as well as benign intracranial lesions have been implicated (5, 6, 7). Restoration of normal blood values has been described after successful removal of the brain tumor. Several cases have been observed in which polycythemia of impressive degree was associated with large uterine myomata (8, 9, 10, 11). After hysterectomy, blood values returned to normal.

A surprising number of cases has been encountered in which polycythemia was associated with tumors of the kidney. Data from 18 of these are included in Table I. Two additional cases are mentioned by Lawrence and his associates (3) but no data are available. Forssell (22) refers to two cases which have not been reported. Through personal contacts with several investigators, we have learned of the existence of 6 other cases, as yet unreported, in which polycythemia was associated with renal neoplasms. The relationship between the blood disorder and the tumor has not been established with certainty. It is quite possible that some patients had polycythemia vera. Enlargement of the spleen was noted in several, but leukocytosis of appreciable degree occurred only once. In our group of cases, in which repeated careful examinations were performed, the absence of leukocyte and platelet abnormalities was impressive. In the one patient who was thought to have splenomegaly, the left upper quadrant mass was subsequently found to be a renal tumor.

In several cases, polycythemia disappeared after nephrectomy. Reappearance of polycythemia in two of these was probably associated with recurrence of the tumor. The most impressive evidence of a causal relationship between renal tumor and polycythemia is provided by the case reported by DeMarsh and Warmington (18). Their patient was a child who had severe polycythemia from infancy. Removal of a benign adenoma of the kidney at age 14 was followed by dramatic subsidence of the polycythemia and the patient was known to be well for more than 5 years after the nephrectomy. The youth of this patient and the apparent recovery make polycythemia vera an untenable diagnosis. It is noteworthy that 2 other patients in the series were under the age of 40 years.

Polycythemia antedated the discovery of the renal tumor in almost all of these cases. In most instances the occurrence of hematuria eventually led to the discovery of the tumor, although bleeding into the urinary tract was sometimes mistakenly attributed to the polycythemia. In some cases hematuria was never observed and the neoplasm was recognized late in the course of the disease or only at autopsy. Many of the patients had been treated by venesection or with ionizing radiation for what was considered to be polycythemia vera. That

TABLE I

Data in 18 Patients with Polycythemia Associated with Renal Tumors

Author	Age, Race, Sex	Blood Values					Spleen Size	Nature and Extent of Tumor	Effect of Nephrectomy	Comment
		Hematocrit	Hemoglobin	Red cell count	White cell count	Platelet count				
		%		*millions/cmm.*	*per cmm.*	*per cmm.*				
1. Medvei (12)	62 WM	—	142%	8.00	11,600	—	To umbilicus	Hypernephroma, right kidney. Metastases to left kidney, liver, retroperitoneal nodes	—	—
2. Fairley (13)	48 WM	—	20.4 Gm.	8.40	8,200	Normal	2 fingers below costal margin	Clear cell adenocarcinoma, right kidney, adherent to diaphragm	Unknown because of chronic anemia due to recurrent bleeding from peptic ulcer.	Polycythemia discovered one year before urinary symptoms. Renal tumor removed 29 months after onset of urinary symptoms. Patient lived for 2 years after nephrectomy.
3. Fairley (13)	36 WM	—	20.7 Gm.	7.00	5,500	Normal	Not palpable. 300 Gm. at autopsy	Anaplastic carcinoma. Small primary in bronchus with extensive metastases to kidneys and other sites	—	Presented with renal colic and hematuria and found to have polycythemia.
4. Forssell (14, 15)	49 WM	—	121%	8.32	4,800	374,000	Not enlarged	Hypernephroma, right kidney later involving left kidney	Polycythemia disappeared. Recurred after 14 months.	Melena due to duodenal ulcer. Later hematuria and polycythemia found. Nephrectomy performed 1 year after onset of hematuria. Recurrence of polycythemia associated with hypernephroma of left kidney.
5. Forssell (16)	71 WF	61	128%	8.15	6,800	185,000	Not enlarged	Tumors of right kidney on pyelography	—	Abdominal pain and hematuria, then polycythemia discovered. No operation or autopsy. Lived 2 years after onset of hematuria.
6. Forssell (16)	66 WM	—	109%	6.00	5,900	Normal	Not enlarged	Hypernephroma, left kidney, with metastases in lungs and adrenal	—	Microscopic hematuria but no urinary symptoms. Died 2 months after first seen.
7. Forssell	65 WM	—	120%	6.18	3,000	Normal	—	Hypernephroma, right kidney with	—	Abdominal pain: hematuria, palpable tumor. Died 8 months later.

								tastases in lungs and adrenal		after first seen.
7. Forssell (16)	65 WM	—	120%	6.18	3,000	Normal	—	Hypernephroma, right kidney with local invasion of surrounding tissue	—	Abdominal pain: hematuria, palpable tumor. Died 8 months later.
8. Videbaek (17)	55 WM	—	147%	8.67	7,600	302,000	Not enlarged	Hypernephroma, left kidney. Metastases to lungs	—	Onset with hematuria. Polycythemia treated with x-ray. Died 13 months after onset.
9. Videbaek (17)	57 WF	—	95%	6.90	70,000	760,000	To umbilicus	Hypernephroma, left kidney, metastasis to liver	—	Polycythemia, hyperuricemia, arthritis of toes preceded hematuria by 5 years.
10. Videbaek (17)	45 WM	—	130%	10.65	5,080	100,000	Not enlarged	Hypernephroma, right kidney	Six months postoperation plethora had subsided and Hgb. 81%, RBC 3.84.	Plethora noted 1 year before onset of hematuria.
11. DeMarsh & Warmington (18)	5 WF	72	23 Gm.	7.6	5,350	—	Not enlarged	Renal adenoma	Complete recovery.	Polycythemia until age 14, when renal adenoma removed. Well thereafter.
12. Gros (19)	53 WM	—	—	7.5	6,800	160,000	—	Hypernephroma	Normal 3 years after operation.	
13. Moe (20)	55 WM	—	140%	7.22	6,600	514,000	Not enlarged	Hypernephroma, right kidney, brain, pleura	—	Presented with pleural effusion and found to have polycythemia. Later developed cerebral manifestations. No urinary symptoms. Died in 3 months. Renal tumor discovered at autopsy.
14. Herbeuval et al (21)	45 WM	—	165%	8.45	8,500	250,000	Palpable	Hypernephroma, right kidney with metastases to lung, mediastinum, aortic nodes	—	Polycythemia for 3 years, treated with radioactive phosphorus. No urinary symptoms, no hematuria, and urographic studies normal. Hypernephroma discovered at autopsy.
15. JHH 400365	61 WM	62	18.6 Gm.	7.20	5,050	210,000	Not en larged	Hypernephroma, left kidney	Unknown.	—
16. JHH 721098	32 WM	70.8	21.5 Gm.	8.64	9,200	280,000	Not enlarged	Hypernephroma, left kidney	No effect, but metastases present.	—
17. JHH 722394	54 WM	—	19.6 Gm.	6.50	12,000	282,000	Not enlarged	Hypernephroma, left kidney	Well one year, then recurrence.	
18. CHH 94378	46 WM	68	—	7.03	5,000	170,000	Not enlarged	Hypernephroma, right kidney	—	

the circulating blood volume must have been greatly increased is indicated by the enormous amounts of blood removed from some of the patients in an effort to control the polycythemia. Review of the case histories makes it clear that a prolonged period of polycythemia preceding the onset of symptoms referable to the urinary tract does not exclude the possibility that the tumor was primary. It is noteworthy that hypertension, sometimes of severe degree, was common. A number of the patients had peptic ulcer, a lesion which is frequently encountered in association with polycythemia vera.

Study of these cases suggests that a renal tumor may in some unknown manner occasionally induce the development of polycythemia. The mechanism by which erythropoiesis is stimulated is obscure. There is no evidence that there is a defect in the oxygenation of the blood. An attractive hypothesis is that the tumor produces a substance with erythropoietic activity. Of interest in this regard are reports that nephrectomized dogs maintained by hemodialysis develop intractable anemia which is not related to the degree of nitrogen retention (23, 24). Evidence has recently been presented which indicates that the plasma erythropoietic factor "erythropoietin" may be produced by the kidneys (25). Quite possibly some renal tumors may produce this or a similar substance in excessive quantities.

SUMMARY

Four cases are described in which polycythemia was associated with hypernephroma. Collateral evidences of polycythemia vera including splenomegaly, leukocytosis and thrombocytosis were not present. It seems quite possible that the polycythemia in these cases was secondary to the renal neoplasm. This point of view is supported by analysis of data from 14 similar cases previously reported. The occurrence of polycythemia in some patients with renal tumors is of special interest in the light of recent experimental observations relating the kidney to the production of an erythropoietic substance.

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INDUCED "IMMEDIATE" HYPERSENSITIVITY TO PURIFIED PROTEIN DERIVATIVE TUBERCULIN (PPD)[1]

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Old Tuberculin (OT), commonly used for diagnostic and epidemiological studies of tuberculosis, is a mixture of broth and bacterial antigens (1). Skin reactions to this material, nevertheless, are usually specific for tuberculosis. However, repeated testing with OT has resulted in development of reactions, probably attributable to induced sensitization, characterized by an "immediate" wheal and erythema or Arthus type hypersensitivity, rather than a typical "delayed" tuberculin reaction (2). Purification of components of the tubercle bacillus has furthered our understanding of immunological phenomena due to this microörganism, and has provided materials of relatively constant biological activity utilizable for clinical testing (3). The development of Purified Protein Derivative (PPD) is of particular importance in this regard.

In the development of PPD, Seibert and her co-workers (6, 7) isolated tuberculo-protein in different stages of purity and with varying degree of antigenicity, but capable of eliciting a typical tuberculin reaction. The PPD available commercially is relatively stable and has a lower molecular weight than other tuberculo-protein derivatives but is somewhat antigenic (3, 6, 7). The antigenicity of PPD is of low order, however, and it has been shown that 10 mg. of the material must be injected repeatedly into a rabbit to produce even moderate "immediate" hypersensitivity skin reactions (7). The author has been unable to find a reported instance of man having developed this form of hypersensitivity on repeated testing with PPD, probably because of its low antigenicity and the extremely small amounts used (0.00002 or 0.005 mg. per injection). The occurrence of "immediate" hypersensitivity to PPD in man would be an important observation and is the basis for this report.

Case report. R. R., a 27 year old white man, was employed in a laboratory working with the tubercle bacillus, but not its fractions, from November, 1954 to September, 1956. His past health had always been good and there was no previous history of allergy, such as hay fever, asthma or urticaria. There was no family history of allergic disease. Because of the nature of this man's employment he received repeated skin tests with different lots of commercially available first and second strength PPD.[2] These tests were always performed on

[1] Supported by Contract DA-18-064-404-CML-100 with the Army Chemical Corps, Fort Detrick, Maryland.

[2] Purified Protein Derivative Tuberculin, Sharp and Dohme, Philadelphia. Various lots. Supplied with sterile buffered diluent.

TABLE I

Skin Tests with PPD

Date	First strength .00002 mg.		Second strength .005 mg.	
	Immediate Reaction	Delayed Reaction	Immediate Reaction	Delayed Reaction
12–7–54	0	0		
12–13–54			0	0
6–13–55	0	0		
6–15–55			0	0
8–22–55	0	0		
8–24–55			0	0
12–12–55	0	0		
12–14–55			0	0
2–29–56	0	0		
3–7–56			0	0
5–23–56	+	0		
6–4–56			+	0
7–18–56	+	0		
7–23–56			+	0
8–15–56	+	0		
8–17–56			+	0

the anterior aspect of the forearms. His first skin test was performed in December, 1954, and over the ensuing 2 years he was tested 8 times (Table I), and received a total of 0.046 mg. of PPD. At no time did he develop a typical "delayed" tuberculin reaction. Beginning with the sixth series of skin tests, he developed an "immediate" wheal and erythema reaction at the site of inoculation, and with each subsequent injection the reaction became more intense, but was not associated with systemic symptoms. The reaction developed within 10 minutes and was characterized by a central urticarial wheal, with pseudopod formation, surrounded by a broad zone of erythema, and associated with intense local pruritus. The pruritus, wheal and erythema persisted about 2 hours and then subsided. Two hundred ml. blood was collected, with sterile precautions, from the antecubital vein 5 to 7 days after each of the last 2 series of skin tests. The serum was separated and stored at 4°C. The patient was tested with 1:100 Old Tuberculin (OT)[3] and the same type wheal and erythema reaction developed. Of interest, is the fact that the patient was the only one of 18 employees in the laboratory who failed to become "tuberculin positive" during the 2 year period of his employment.

EXPERIMENTAL METHODS AND RESULTS

Analysis for serum precipitin. An analysis for precipitin was performed by adding 0.4 ml. of the patient's undiluted serum to 0.4 ml. of PPD[2] in concentrations varying from 0.1 to 0.006 mg./ml. buffered diluent. These were agitated and incubated at 37°C. for 2 hours, then placed at 4°C. for 5 days. A similar analysis was carried out with tuberculo-polysaccharide.[4]

[3] Old Tuberculin, prepared by the Allergy Clinic, the Johns Hopkins Hospital.

[4] Kindly supplied by Dr. Florence B. Seibert, Henry Phipps Institute, University of Pennsylvania, Philadelphia. Tuberculopolysaccharide I. (0.27 per cent nitrogen).

No precipitating antibody was detected in the patient's serum, under the conditions of this experiment.

Passive transfer of hypersensitivity with serum. Five normal, "tuberculin-negative", adults were injected intracutaneously into 2 areas of the anterior forearm with 0.2 ml. of the patient's serum. Twenty-four hours later one of these inoculated sites was challenged with 0.1 ml. of first strength PPD (0.00002 mg.), and the second site was injected with the buffered diluent. A third site, not prepared with serum, was challenged with first strength PPD, as control.

Each of the challenged persons developed an immediate wheal and erythema reaction at the site of serum injection, following inoculation of PPD. These reactions were identical with those observed in the patient. Therefore, it was possible to passively transfer with serum the "immediate" hypersensitivity to tuberculin.

The development of "immediate" hypersensitivity to PPD may possibly have prevented the occurrence of a typical "delayed" tuberculin reaction in the patient. To evaluate this, the following experiment was performed.

Two "tuberculin-positive" adults were tested with first (0.00002 mg.) and second strength (0.005 mg.) PPD to confirm the presence of "delayed" hypersensitivity. One of these subjects reacted to first strength PPD, and the other to second strength PPD. After this determination, both persons were inoculated intracutaneously with the patient's serum and then challenged with PPD in the same way as in the preceding experiment. First and second strength PPD, respectively, were inoculated into the serum prepared site of each recipient.

Both subjects developed wheal and erythema reactions and 48 hours later had "delayed" tuberculin reactions. Therefore, it was shown that the "immediate" hypersensitivity to PPD did not prevent the "delayed" tuberculin reaction.

Hemolysis of tuberculin-sensitized erythrocytes by the patient's serum. This analysis was kindly performed by Dr. Curtis Williams in the laboratories of the Rockefeller Institute for Medical Research, N. Y.

Sheep erythrocytes were "sensitized" by incubation of 0.5 ml. packed cells with 25 ml. diluted Old Tuberculin (Lederle) for 2 hours at 37°C. Serum complement and the patient's heat "inactivated" serum were absorbed with normal sheep erythrocytes prior to testing. The patient's absorbed serum was serially diluted and to 0.5 ml. of each dilution, 0.05 ml. of guinea pig complement (1:2) was added. To these mixtures 0.5 ml. of 0.5 per cent "sensitized" cell suspension was added. Suitable controls of "sensitized" erythrocytes in buffer and complement, and unsensitized cells in antiserum diluted 1:10 were performed. The tubes were placed at 4°C. for one hour, and 37°C. for 15 to 30 minutes, or until lysis occurred with a standard positive serum. The

highest dilution of the serum producing hemolysis was considered the "serum titer".

The hemolysin titer of the patient's serum was 1:160. This indicates that associated with the "immediate" hypersensitivity to PPD there was serum antibody capable of causing lysis of erythrocytes sensitized with Old Tuberculin.

Determination of the component of PPD responsible for the "immediate" hypersensitivity. a. Twelve guinea pigs weighing approximately 300 gm. were inoculated intracutaneously with 0.1 ml. of undiluted and diluted (1:2) serum on alternate sides of the back. Six hours later half of the animals were injected intravenously with 0.1 mg. PPD in 1 ml. of 0.5 per cent Evan's blue dye. At the same time, the remaining 6 animals were injected intravenously with 0.1 mg. tuberculo-polysaccharide.[4] Thirty minutes later the skin sites were inspected, the animals killed with ether, and the skin was removed.

Three of the 6 guinea pigs given tuberculo-polysaccharide showed positive passive cutaneous anaphylaxis reactions (11), indicated by the accumulation of blue dye at the skin sites inoculated with undiluted and diluted serum. One of the animals given PPD showed a faint reaction at the skin site injected with undiluted serum. It was, therefore, possible to passively transfer the patient's "immediate" hypersensitivity with serum to guinea pigs, and the hypersensitivity was particularly directed against the tuberculo-polysaccharide.

b. If tuberculo-protein is digested with trypsin it loses its ability to elicit a "delayed" tuberculin reaction (3). Therefore, to determine if trypsin treatment of PPD would similarly destroy the ability of the material to elicit a wheal and erythema reaction in passively sensitized human skin, the following experiment was performed.

Crystalline trypsin[5] was dissolved in buffered diluent in a concentration of 0.01 mg./ml. One-tenth ml. of this was added to 0.0002 mg. PPD dissolved in 0.9 ml. of diluent. The mixture was incubated at 37°C. for 2 hours, and 0.1 ml. of this was inoculated into the skin of a "tuberculin-positive" recipient who had been injected with 0.2 ml. of the patient's serum 24 hours previously. One-tenth ml. of non-trypsinized PPD, diluted and incubated in the same way, was inoculated into a similarly prepared skin site of the forearm.

An acute erythematous pruritic reaction developed at the site of injection of trypsin-treated and untreated PPD. This reaction faded in about 2 hours and was followed 24 hours later by the appearance of a *faint* "delayed" tuberculin reaction only at the site challenged with untreated PPD. Therefore, the ability of PPD to elicit a typical "delayed" tuberculin reaction was eliminated by treatment with trypsin, but an "immediate" hypersensitivity reaction to this material was produced.

[5] Baltimore Biological Laboratories, Baltimore.

DISCUSSION

The Purified Protein Derivative Tuberculin prepared by Seibert and Glenn (7), which is the standard for the commercially available PPD, contains about 6 per cent polysaccharide and 93 per cent protein. Therefore, the amount of polysaccharide given to the patient in this study, *prior* to development of his "immediate" wheal and erythema hypersensitivity, was approximately 0.0015 mg. The amount of protein given was about 0.0235 mg. Repeated inoculation of 0.5 mg. PPD will not induce hypersensitivity in rabbits, although 10 mg., given repeatedly, will elicit Arthus-type reactions (7). This amount of tuberculin is greatly in excess of that used for skin testing in man, and development of "immediate" hypersensitivity to PPD by repeated inoculation must be a rare occurrence in the human.

The "immediate" hypersensitivity induced in the patient reported was passively transferred with serum to normal and "tuberculin-positive" recipients, indicating that the sensitization was due to formation of serum antibody.

Previous investigators (4, 8) have been unable to stimulate antibody formation or hypersensitivity in experimental animals by inoculation of purified tuberculo-polysaccharide. However, tuberculo-protein may be antigenic (7). Therefore, it might be assumed that the induced hypersensitivity to PPD, described here, is attributable to the protein fraction. But the ability of trypsin-treated PPD to elicit a wheal and erythema reaction following passive sensitization suggests that the tuberculo-polysaccharide was the sensitizing agent. Furthermore, the ability to elicit passive cutaneous anaphylaxis particularly with purified tuberculo-polysaccharide supports the suggestion that this is the sensitizing antigen.

A possible explanation for the failure of the patient to develop a "delayed" tuberculin reaction similar to that of other personnel working in the same laboratory might have been an inhibitory effect of the induced sensitization on the "delayed" hypersensitivity. However, the serum antibody induced in the patient by repeated inoculation of PPD was incapable of blocking the "delayed" reaction of "tuberculin-positive" recipients. This is of practical importance because it indicates that one need not be concerned about an "immediate" hypersensitivity to PPD in the interpretation of the tuberculin-reaction as a criterion of tuberculous infection.

The patient had a positive tuberculin-hemolysin titer, which is of interest in this instance by its not being associated with a positive "delayed" tuberculin reaction. It is impossible to state definitively whether antibody to the tuberculo-protein or tuberculo-polysaccharide was responsible for this finding. However, Keogh and his co-workers (10) have shown that tuberculo-polysaccharide can be adsorbed to normal erythrocytes while tuberculo-protein cannot. This suggests that the fraction of the PPD responsible for sensitization and de-

velopment of the positive hemolysis test may be the polysaccharide component of the Purified Protein Derivative.

SUMMARY

A "tuberculin-negative" adult male is described who developed an "immediate" wheal and erythema hypersensitivity to commercially available Purified Protein Derivative Tuberculin (PPD) after repeated skin testing over a two year period. The patient's hypersensitivity to tuberculin was passively transferred with serum to "tuberculin-negative" and "tuberculin-positive" recipients, and elicited passive cutaneous anaphylaxis in guinea pigs. The "immediate" hypersensitivity reaction passively transferred to "tuberculin positive" recipients did not appear to inhibit development of the typical "delayed" tuberculin reaction. Associated with the "immediate" hypersensitivity to PPD there was a high titer of serum hemolysin for tuberculin-sensitized sheep erythrocytes. Trypsin-treatment of PPD abolished the ability of the material to elicit a "delayed" tuberculin reaction but did not prevent it from causing an "immediate" hypersensitivity reaction, and the reactivity of passively sensitized guinea pigs was particularly directed to tuberculo-polysaccharide. These findings are discussed and it is suggested that the induced sensitization may be attributable to the polysaccharide content of the PPD.

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HITHERTO UNDESCRIBED CHARACTERISTICS OF THE PATHOLOGY OF INFANTILE CORTICAL HYPEROSTOSIS (CAFFEY'S DISEASE)

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INTRODUCTION

In 1945 Caffey and Silverman (1) described a new syndrome or disease in infants, characterized by sudden swelling of the soft tissue overlying a bone and, subsequently, periosteal new bone production in the bone at the site of the soft tissue swelling. The lesions are painful. With rib involvement pleural effusions frequently occur. Multiple bones are involved frequently, but not always, and the soft tissue swelling, if not the bone, fluctuates in size. The x-ray of the involved site shows, in the fully developed lesion, non-specific subperiosteal thickening of the bone, but no calcification of the soft tissues. In the early stages little or no enlargment of the bone is seen on x-ray, but the soft tissues are swollen. Systemic signs of fever, leucocytosis, anemia, and irritability usually are present. The disease runs a self-limited course and, in most cases, the enlarged bones return to normal in a few months as judged clinically and radiologically. However, persistent deformity of a bone occurs rarely (1). No infectious agent has been identified by culture of the biopsy specimen or blood, although the clinical course suggests an infection.

The syndrome was called originally, Infantile Cortical Hyperostosis, and now is widely known also as Caffey's disease. While the original clinical and radiological observations have been widely substantiated, there being 71-odd cases in the literature, the lesions have not been studied histologically in many cases.

The pathological process, as described in the few cases which have been biopsied, shows subperiosteal new lamellar bone formation without evidence of inflammation or hemorrhage. The periosteum is described as thickened and hyperplastic and the soft tissues as edematous (1, 2, 3, 4, 5, 7, 9). However, "inflammatory infiltration of the bone" (6), "round cells in the adjacent muscle" (8), and "chronically inflamed tissue" (10) have been reported.

The purpose of this report is to show that a hitherto undescribed *acute* inflammation occurs at an early stage of the disease, and that this is associated with loss of the periosteum and occasionally of the compactness of the subperiosteal layers of the original bone as definite histologic structures. Simultaneously there is a marked fibrous and osteoblastic reaction with much osteoid

deposition in the loose fibrous tissue surrounding the bony trabeculae. This whole process extends out to invade the surrounding soft tissue including the muscle. Therefore the pathological process is intra-periosteal and inflammatory in its early stages rather than subperiosteal and non-inflammatory as the reported cases describe. Eventually the inflammation subsides spontaneously and the inflamed callus-like process becomes organized into normal trabecular bone which is covered by a thickened periosteum from which scar tissue extends out into the soft tissues. At this *later* stage a subperiosteal cap of lamellar bone, without inflammation, is seen. This is the stage of the disease that has been repeatedly described in biopsies.

In addition to the second case, which was serially biopsied, six other cases from our surgical pathology files are presented.

Case I (W. G.)

A colored male infant, age 2 months, was examined at The Children's Hospital School on August 20, 1950 with a one week history of painful swelling of his left scapula. The shoulder area had gradually enlarged during the preceding week.

He was admitted to The Children's Hospital School on August 30, 1950. There was a mild anemia, the hemoglobin being 8.5 grams. The urine was normal. The white blood count was 9200, and the serum proteins and phosphorus were normal. The rectal temperature was 101 F. and he continued to run a low grade fever until operation.

X-rays showed enlargement of the scapula with a reduplication of the scapular cortex. At some points the original cortex of the scapula and the left clavicle were indistinct, and this suggested a lesion surrounding the bone that might be both lytic as well as osteoblastic.

On September 5 a biopsy was taken because the scapula had about doubled in size in the two weeks of observation since August 20. This tiny biopsy was diagnosed as "osteoblastic reaction, ? tumor." After much discussion and a thorough review of the biopsy material a shoulder girdle arm amputation (S.P. 51-4043) was performed, a month following the original biopsy. Assurance could not be given the surgeon that this was not an osteogenic sarcoma.

The postoperative course was uneventful, and when last seen in 1954 the patient was well and there was no evidence of tumor recurrence or metastasis. The clinical course following shoulder girdle amputation and the similarity to the second case both from the standpoint of the history, physical examination and biopsy material leads to the conclusion that this patient actually had Caffey's disease of the left scapula and clavicle.

(S. P. 51-4043, figs. no. 1, 2, and 3) Examination of the gross amputation specimen showed that the left arm and hand were normal. The scapula was enlarged posteriorly where a mass 6 by 8 by 6 cm. protruded. It was composed of "soft bone" which could be easily cut with a knife. The surrounding soft tissue was pale and in it there appeared to be a "gelatinous edema."

Microscopic examination of the lesion on the scapula showed the bone to be normal except for loose fibrous tissue filling the marrow spaces. Only a few marrow cells remained. The tissue interpreted as periosteum was very hyperplastic being several times normal thickness and was composed of fibrous tissue cells many of which appeared to be osteoblasts. Much osteoid was being formed in a cellular fibrous stroma in and between this hyperplastic periosteum and the original bone, and the whole process of osteoid formation was extending out to invade the surrounding soft tissue and skeletal muscle. Numerous inflammatory cells,

some round cells, but mostly polymorphonuclear leucocytes were seen infiltrating the fibrous tissue. Associated with the newly formed and as yet uncalcified osteoid were many purulent foci. No bacteria were stainable in these. The original "cortex" of the bone was not easily found, although in places it could be seen underlying the new bone formation. "Protein-rich edema fluid" in which were some fibroblasts and inflammatory cells separate the skeletal muscle fibers external to the bone. No hemorrhage or hemosiderin were seen. The lymph nodes were very edematous.

Case II (M. B., HLH #B-16193)

This 5 week old colored male was first seen on December 16, 1954 in the Out-Patient Clinic of The Johns Hopkins Hospital with a history of a flaccid left arm since birth. This was considered to be an Erb's palsy until on examination in the Orthopaedic Clinic it was pointed out that the patient could move the arm when necessary to withdraw it from painful stimuli such as pin prick of the skin. There was also apparently tenderness of the enlarged left scapula.

There was nothing in the birth history or development to suggest trauma. The temperature was normal rectally (37.5°C). WBC count was 15,700, Hgb. was 9.6 grams per 100 c.c., ESR was 21 corrected. The old tuberculin skin test, the STS, and a sickle cell preparation of the blood were all negative. Two blood cultures were sterile. Serum calcium and phosphorus were normal.

Initial x-rays of the skeleton demonstrated no lesion except that in the left scapula. The scapular outline was indefinite and a soft tissue density greater than that of muscle surrounded the scapula.

The possibility of a tumor of the scapula was considered, but due to the experience noted under Case I it was realized that the findings were compatible with Caffey's disease.

Biopsy of the scapula was performed December 21, 1954 (S.P. 54-5986). Frozen section of the non-ossified peripheral part of this biopsy specimen gave the impression of "? fibrosarcoma". However, amputation was not carried out. Study of the whole biopsy specimen which included part of the original scapular bone as well as the non-ossified peripheral outgrowth of the scapula was made. The child withstood the procedure well, except for a mild febrile response after operation.

The biopsy was sterile on culture and smears were negative for bacteria and fungi. On January 20, 1955, about one month postoperatively, it was possible by x-ray to detect involvement of this patient's left clavicle and both mandibles. Furthermore, the hazy soft tissue shadow which had surrounded the scapula originally and which on biopsy proved to be non-calcified osteoid was now definitely calcified and had the appearance of new bone. The whole picture therefore seemed typical of infantile cortical hyperostosis by x-ray examination and clinical course.

The patient was readmitted on February 9th, 40 days after the original biopsy, at 3 months of age, for a repeat biopsy of the left scapula. At that time the portion of the scapula removed included both the new bone and part of the original scapula as did the original biopsy. It was taken in an area removed far enough from the original biopsy so that changes secondary to the original operation were not likely to be present (S. P. 55-713). This specimen was also sterile for bacteria and fungi.

The postoperative course was benign. By May 3, 1955, the scapula, clavicle and mandible were shrinking in size both clinically and by x-ray, and by July 6, 1955, the child was discharged as recovered.

(S.P. 54-5986, figs. no. 4, 5, 6, 7, and 8). The *first* biopsy shows on microscopic examination the bony trabeculae in the calcified cortex of this infant scapula to be more numerous

than normal and a loose fibrous tissue fills the marrow spaces. At the periphery of the original scapula cortex, the osteoblasts are very numerous around the trabeculae and the marrow spaces between these are filled with a loose fibrous tissue. As one proceeds peripherally from the cortical bone where a periosteum should be seen one finds instead extremely cellular fibrous tissue throughout which osteoid formation is rampant extending out to invade the adjacent skeletal muscle. This osteoid peripheral to the original bone is undergoing calcification in an irregular manner and numerous osteoclasts are seen adjacent to the osteoid. Scattered through the cellular fibrous tissue and osteoid and extending out into the adjacent soft tissue are many polymorphonuclear leucocytes, and these form numerous small purulent foci. Bacterial stains show no organisms.

(S.P. 55-713, figs. no. 9, 10, and 11). The *second* biopsy, 40 days after the first one, shows a cap of less mature bone overlying older appearing bone. The newer bone appears to be located where very incompletely calcified osteoid was found in the original biopsy. The stroma of vascularized fibrous tissue which occupies the marrow spaces between the trabeculae contains a few lymphocytes and macrophages. In places osteoid is seen in the fibrous stroma, but most of the osteoid is now well calcified. Numerous osteoclasts are seen adjacent to some of these trabeculae suggesting inception of the remodelling process. A thick cellular periosteum is now observed as a definite structure peripheral to this new bone. Strands of fibrous tissue extend out from this periosteum into the adjacent skeletal muscle.

Case III. (R. R., Hist. No. A-34238).

A 3 month old white female was first seen on February 22, 1944, in the out-patient clinic with a one month history of facial swelling and intermittent low grade fever. Birth history and development were normal.

On examination she was irritable with a puffy, rather indurated swelling over the right side of the mandible. When seen again on February 27, she had developed swellings of both lower legs and was admitted to the Harriet Lane Home of The Johns Hopkins Hospital.

Laboratory findings revealed a hemoglobin of 12 grams, a white blood count of 22,000 with 68 per cent polymorphonuclear neutrophils. Her temperature was normal (37.5°C). Urinalysis, old tuberculin skin test, serological test for syphilis, and blood culture were negative. The roentgenograms taken on March 11 were interpreted as showing normal mandibles and tibiae, although when the x-rays were reviewed in 1950, they were rediagnosed as showing the characteristic picture of infantile cortical hyperostosis. The swelling persisted and on March 16 a mandibular biopsy was performed, and this was sterile on culture. (S.P. 88101).

Films on March 29, 1944 were said to be normal and the course of the child from then on was benign. She was seen at age 10 months with normal dentition and no evidence of disease, clinically or radiologically. The rapid remodelling of bone in the first six months of life is well demonstrated by these observations.

(S.P. 88101). The microscopic section shows extensive osteoid deposition in a loose fibrous tissue stroma which fills the marrow spaces, and scattered through this are numerous polymorphonuclear leucocytes and round cells. Osteoblasts are not prominent, and no osteoclasts are seen. The bony trabeculae are much thicker than normal. No hemorrhage or hemosiderin are present. The biopsy is small, and no cortical bone or soft tissue is present.

Case IV. (G. S., Hist. No. B-03224).

This colored male was seen in the Harriet Lane Home of The Johns Hopkins Hospital Out-Patient Clinic at age 8 months on July 3, 1953. He had had an intermittent swelling of the left jaw for 4 months. This had been associated with anorexia and vomiting.

Examination showed diffuse enlargement of the left mandible and cheek. Roentgenogram showed enlargement of the left mandible and this was interpreted as cortical hyperostosis. The soft tissues were also noted to be swollen.

He was followed as an out-patient and on October 14 he had another episode of swelling of the left mandible. The mass appeared to be enlarging and the skin was reddened and tender. The temperature was 38.5°C. This enlargment lasted one week, then the mass decreased in size. On February 26, 1954, the mass again increased in size, and, when the teeth erupted, the left jaw became greatly enlarged. At this time, at the age of 18 months, he was admitted to the hospital.

The hemoglobin was 12.6 grams with a erythrocytic sedimentation rate corrected to 4. White blood cell count was 12,700 and urinalysis, old tuberculin skin test and serology were negative. Serum calcium, phosphorus, and alkaline phosphatase were normal.

The radiograph at this time showed rather extensive cystic change in the left mandible not characteristic of infantile cortical hyperostosis (Fig. 15). With a clinical diagnosis of probable neoplasm he underwent an operation, the left half of the mandible being removed. (S.P. 54-1686).

He was discharged to return for plastic reconstruction of his mandible. No other bone lesions were noted during the following two years.

(S.P. 54-1686, figs. no. 12, 13, and 14. The gross specimen consisted of the left half of the mandible which was markedly enlarged laterally where it measures 2.8 cm. in thickness. Proximal and distal to this it was normal in appearance. Microscopic sections show thickening of the bony trabeculae, and these are more numerous than normal. A new layer of trabecular bone has formed over the external limits of the original bone much of the latter having been destroyed. A thickened fibrous periosteum forms the outer margin of the new bone and fibrous tissue extends out from this to involve the adjacent soft tissue. Around the spicules of bone osteoblasts are prominent, and in places they form solid nests. In other areas osteoclasts are numerous, usually being adjacent to bone, and here the bone has a moth-eaten appearance. A very loose fibrous stroma replaces the marrow spaces and numerous inflammatory cells, mostly polymorphonuclear leucocytes, widely infiltrate this. In places, there are purulent foci and in some of these, giant cells thought to be osteoclasts are seen. The trabeculae in such areas are destroyed, which probably accounted for the cystic appearance in the x-rays. No bacteria can be seen in appropriate stains in such foci. No hemosiderin or other evidence of hemorrhage is seen. The lymph nodes from the submandibular area show extensive follicular and lymphoid hyperplasia.

Case V. (P. B., Hist. No. A-78671)

This child was seen at 2½ months of age, following a normal pregnancy and normal subsequent development. Her face became swollen on the left two days before being seen on June 15, 1950. Her temperature was elevated to 38°C. The mandible was swollen bilaterally, but was thought to be non-tender.

She was anemic, having 8 grams of hemoglobin, and a leucocytosis of 16,000, 45 per cent being polymorphonuclear leucocytes. Blood cultures were sterile on three occasions. Serum calcium and phosphorus were normal. Urinalysis and serological test for syphilis were negative.

On June 16, x-rays of her mandible showed periosteal "layering" typical of infantile cortical hyperostosis. On June 19, a biopsy of the mandible was performed which was sterile on culture and negative on smear for bacteria and fungi. (S.P. 50-2555).

Her hospital course was benign except for some tenderness of the mandible on her second hospital day.

Following discharge she was followed in the out-patient clinic and on August 8, 1950 was clinically normal. X-ray evidence of mandibular "layering" had disappeared, but the mandibles were still thickened. No other bone lesions developed.

(S.P. 50-2555, figs. no. 16 and 17). The biopsy shows on microscopic examination a cap of new trabecular bone overlying the original bone, and portions of the cortex can be seen between these, but it has been destroyed for the most part (Fig. 16). The osteoblasts in the outer shell of bone are prominent, lining the trabeculae and, in places, forming small aggregates. Mostly, but not exclusively, in such accumulations one sees in the osteoblast a large vacuole, containing an ill-defined reticulum, which pushes the nucleus peripherally (Fig. 17). Only rare osteoclasts are seen. A very loose fibrous stroma fills the marrow spaces, and only rare marrow cells are seen. No inflammatory cells are present. A hyaline periosteum separates the bone from the adjacent skeletal muscle and a thin cortex is present under this. The muscle from the area of the biopsy shows extensive scarring and in the scar the muscle fibers are atrophic.

Case VI. (P. R., Hist. No. B-21292)

This 4 month old colored female was born at home and was normal until at 3 months of age a swelling developed over the left shoulder. At that time the shoulder was tender but radiographs were negative. On July 28, 1955, at age 4 months, she was seen in the Harriet Lane Home of The Johns Hopkins Hospital with a diffusely enlarged and thickened left scapula, which was non-tender and did not interfere with motion of the arm.

The child's hemoglobin was 9.9 grams and the sedimentation rate and white blood count were normal. Other examinations including old tuberculin skin test, serological test for syphilis, urinalysis and serum calcium and phosphorus were normal.

The roentgenograms were compatible with the diagnosis of infantile cortical hyperostosis involving only the scapula. Biopsy was performed on August 1, and this was sterile on culture. (S.P. 55-3678). The child's hospital course was uneventful and on August 25, 1955 the x-ray showed evidence of resolution of the abnormal bony overgrowth of the scapula. No further lesions developed.

(S.P. 55-3678, fig. no. 18). The microscopic section consists of cancellous bone. The trabeculae are more numerous and thicker than normal, but consist of well organized bone. A loose fibrous stroma fills the marrow spaces where a few chronic inflammatory cells and rare marrow cells are seen. The osteoblasts around the trabeculae are numerous, and, in places, occur in small clumps. Their cytoplasm is basophilic and many contain a cytoplasmic vacuole in which a reticular network is seen. The vacuole is large, pushing the nucleus peripherally (Fig. 17). The osteoclasts are numerous and normal in appearance.

Case VII. (W. R., Hist. No. B-18673).

A 2 month old colored male was first seen in the out-patient clinic on March 22, 1955. He had had swelling of both jaws for 4 weeks and swelling of the right leg for 3 weeks, and was very irritable with marked anorexia and intermittent fever. There was nothing remarkable in his past history.

On the initial examination swelling of the jaws, right tibia and ribs was noticed. The child was ill and appeared emaciated. The swollen areas were hot and tender. The right inguinal nodes were markedly enlarged.

He was admitted March 27, 1955, at which time his physical examination was unchanged except for increase in size and tenderness of the previous lesions as well as a new swelling in the right scapula.

His hemoglobin was 9.6 grams with a leucocytosis of 39.700, 73 per cent being poly-

morphonuclear leucocytes. Other routine laboratory work including blood cultures, serum calcium and phosphorus, serological test for syphilis, old tuberculin skin test and urinalysis were negative.

The x-rays showed extensive involvement of the ribs, right tibia, right fibula, both humeri, both sides of the mandible, the orbital plate, and lesser sphenoid bones of the cranial vault. The right tibia and fibula were actually pushed apart by the peripheral osteoid forming on these two bones. At the time of the original examination this osteoid was so incompletely calcified, that is, absorbed very little x-ray and so was not of a calcific density.

On March 30 biopsies of the right tibia and inguinal lymph nodes was performed (S.P. 55-1513) which on routine culture for bacteria and fungi were sterile. Lymphoid tissue and bone were cultured for virus by tissue culture techniques. A virus, of as yet undetermined type, was cultured from the bone. This material is still under investigation. By March 31 the lesions had progressed by radiographic study and the child was still ill. Accordingly he was given cortisone 100 milligrams a day, by mouth, for 10 days. The child became afebrile, but otherwise this treatment did not appear to influence the enlargment of the bones.

He was discharged in fairly good general condition although the swellings persisted. At home he became less irritable and began to gain weight. The lesions in the ribs, mandibles, cranium, scapula and clavicles regressed, but by June 28 the right leg had become more swollen and x-rays showed a synostosis between the shafts of the tibia and fibula. This state still persisted when last seen in October 1955, 7 months after the onset of his disease.

The biopsy (S.P. 55-1531) shows on microscopic examination a layer of trabecular bone to have formed over the original bone and no true smooth cortex is seen. A more compact than normal connective tissue stroma fills the intertrabecular spaces and osteoblasts line the margins of the trabeculae more prominently than normal in the new peripheral bone. Beneath a thick, hyaline, periosteum there is great proliferation of osteoblasts which form solid masses and, in places, a little uncalcified osteoid is seen adjacent to these cells. No osteoclasts are observed. The scarred, thickened periosteum extends out to involve the adjacent adipose tissue. No hemosiderin or inflammatory cells are seen.

DISCUSSION

In Case II a marked change in the histological picture occurred in the interval of forty days between the two biopsies. The pathological picture of the first biopsy differed radically from the few published descriptions of biopsies or autopsies of infantile cortical hyperostosis (1–10). Rather than non-inflammatory subperiosteal new bone production which the various authors describe, there is loss of the periosteum with inflammatory involvement of the intertrabecular bone and overlying soft tissue. While most authors describe the lesion as non-inflammatory, chronic inflammation of the adjacent soft tissues and the bone itself have been briefly mentioned (6, 8, 10). The first four cases presented here show a marked inflammatory reaction with many polymorphonuclear leucocytes, diffuse through the connective tissue in the bone and soft tissue, as well as aggregates forming purulent foci. The bone about such foci is destroyed and this process was so marked in Case IV that small cystic areas were seen in the x-ray (Fig. 15). No stainable bacteria were found in these purulent foci. Similar to the experience of others (3, 11), the soft tissues were very edematous, particularly in Case IV.

In the second biopsy of Case II the character of the lesion had changed radically during the intervening forty days. The callous-like reaction consisting of cellular connective tissue containing much osteoid and numerous acute inflammatory cells involving the bone and adjacent soft tissue as seen in the first biopsy had been replaced by bony trabeculae, increased in number in the deeper bone, and forming a cap of new overlying bone. The inflammation had subsided and a fibrous periosteum surrounding the bony tissue had again become a distinct histologic structure. A thin but relatively continuous cortex of bony tissue separates the bone and soft tissue. Scar tissue extending out from the periosteum involves the soft tissue and a loose vascularized fibrous tissue fills the marrow spaces.

This *second* biopsy of Case II fits admirably the ordinarily described pathology of this disease, namely, non-inflammatory subperiosteal new bone production and scarring of the surrounding soft tissue and marrow spaces. Had biopsies been obtained earlier in the development of the process in the reported cases a picture similar to the first biopsy of this case probably would have been found.

Cases I, III, and IV are also in the inflammatory stage of the disease, and it is unfortunate that serial biopsies were not obtained in these.

It is clear that more than the periosteum and cortex are involved. The marrow spaces are scarred and the trabeculae at appreciable distances from the cortex, or indeed, of a major portion of the bone, are thickened and increased in number.

Importance has been attached to the finding of intimal proliferation of small arteries in the scarred soft tissue about the bone lesions, the idea being that narrowing of these vessels by the thickened intima produces "hypoxia" of the bone which stimulates bone production (3, 5 and 11). In the early lesions described in this report no such narrowed vessels were seen. However, in scars wherever they occur, the arteries do undergo intimal proliferation and this is secondary to scarring and/or inflammation. The changes in the vessels in the reported cases undoubtedly are secondary to the scarring and are not the cause of the process.

The cystic changes seen in the later x-rays of the mandible in Case IV have not been described in Caffey's disease (Fig. 15). These small cysts, under the microscope, are found to consist of collections of polymorphonuclear leucocytes, in places associated with osteoclasts and in such foci the trabeculae are destroyed (Figs. 13 and 14). Such foci contain no stainable bacteria. These foci differ only in size from those seen in Cases I and II. Also, the clinical picture in Case IV was typical of this disease.

Cases V, VI and VIII show no inflammation and it is probable at another stage in their development an inflammatory reaction would have been present.

The vacuoles in the osteoblasts seen in Cases V and VI (Figs. 17 and 18) are not uncommonly seen in very active osteoblastic reactions (12).

Two of these Cases (I and IV) underwent radical amputations with the mistaken diagnosis of tumor, even though Case I had been biopsied. The numerous large connective tissue cells with osteoid deposition about them can easily be mistaken for neoplasm on a small biopsy if one did not have Caffey's disease in mind. However, the incidence of primary bone neoplasm in infants of this age group is extremely low, Caffey's disease being much more common in our experience. The biopsy should include overlying soft tissue, tissue containing the new formed bone, and some of the original bone, all in continuity; or in other words, in the one piece of tissue obtained for histological examination these various parts should be included so that the transitional phases of the lesion can be identified in relation to one another.

Caffey's disease is practically always self-limited and the bony enlargments usually disappear by remodelling in a few weeks or months. Only one death thought to be definitely due to this disease is reported in the literature (5). Biopsy is not necessary if the diagnosis can be made by clinical and x-ray methods. However, there is no contraindication to biopsy if the diagnosis cannot be made by less direct methods. When a biopsy is obtained in an early stage of Caffey's disease, the authors hope that the information presented in this study will help to lead to its correct histologic interpretation and lead away from the diagnosis of a malignant tumor.

SUMMARY

Histological findings of infantile cortical hyperostosis which have not been described previously are presented in this paper. Previously, only non-inflammatory subperiosteal new bone formation has been described. However, it is now clear that in the early stage of Caffey's disease there is a marked acute inflammatory reaction with many polymorphonuclear leucocytes forming small purulent foci in the connective tissue stroma of the abnormally active periosteum. Osteoid trabeculae appear throughout the overactive periosteum in this acute stage of the disease and during this stage the periosteum as a histologically identifiable structure surrounding the bone is thereby lost. The entire process extends out to invade the soft tissues overlying the periosteum, and in foci the peripheral layers of the original bones are resorbed. In this acute stage a biopsy including only the periphery of the lesion may be mistaken for osteogenic sarcoma.

In the subacute stage, the periosteum reforms as a peripherally limiting structure about the old and newly formed bone. In this later stage the osteoid laid down in the acute stage becomes more completely calcified and is "subperiosteal".

In a still later stage or the remodelling stage the extra bone peripheral to the original bone is removed.

The subacute and remodelling stages from the histological viewpoint are the ones which have been previously described in the literature.

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EXPLANATION OF ILLUSTRATIONS

(Photomicrographs by Miss Patricia Whitney)

FIG. 1 (Case I): Low power magnification shows destruction of the cortex and extension of the process out into the soft tissues.

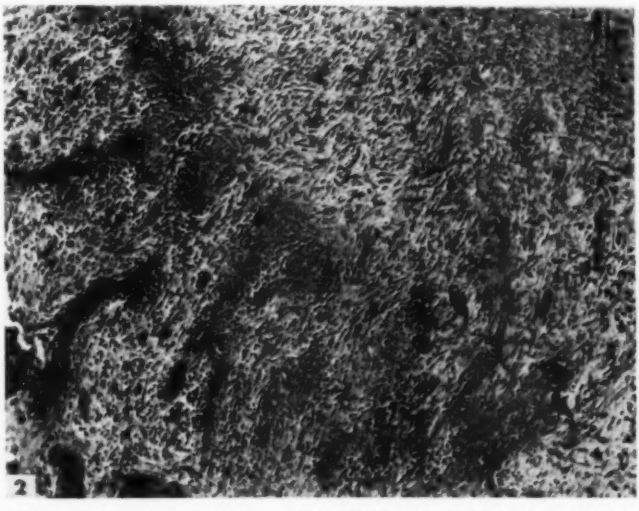

FIG. 2 (Case I): Higher lower shows the cellular connective tissue containing much osteoid.

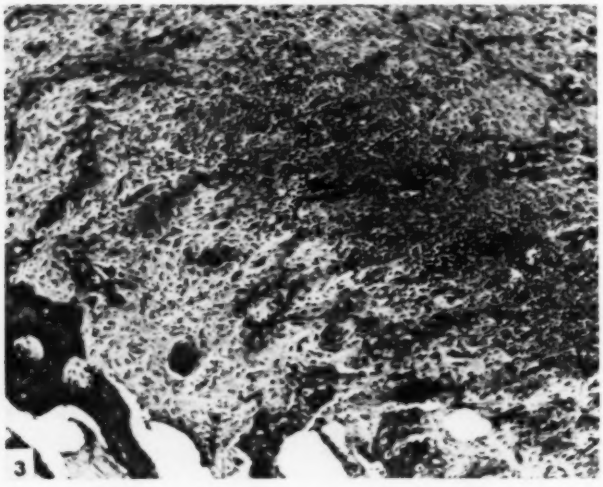

FIG. 3 (Case I): Numerous polymorphonuclear leucocytes infiltrate the fibrous tissue stroma.

FIG. 4 (Case II): Low power magnification shows destruction of the cortex and periosteum with extension of the process into the soft tissues.

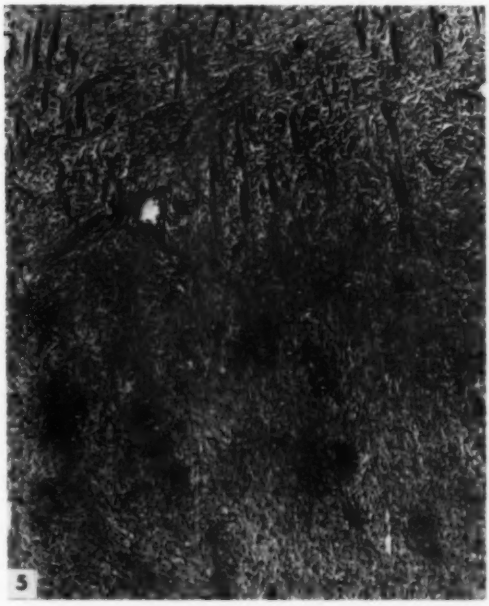

FIG. 5 (Case II): Low power magnification shows the cellular reaction invading the skeletal muscle and separating the fibers. The vertical strands are skeletal muscle fibers.

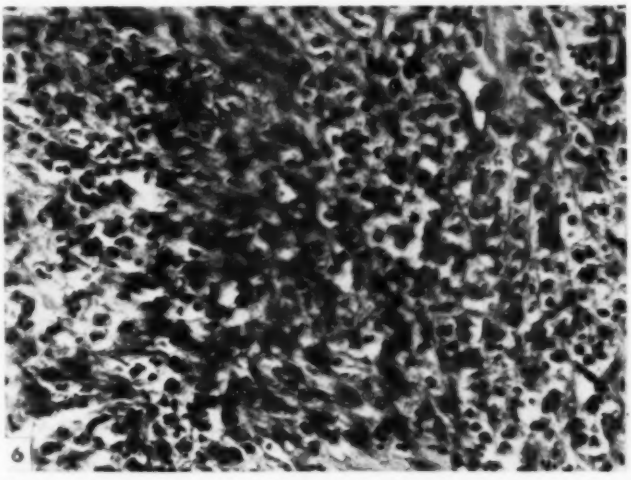

FIG. 6 (Case II): High power magnification shows numerous polymorphonuclear leucocytes in a cellular fibrous stroma.

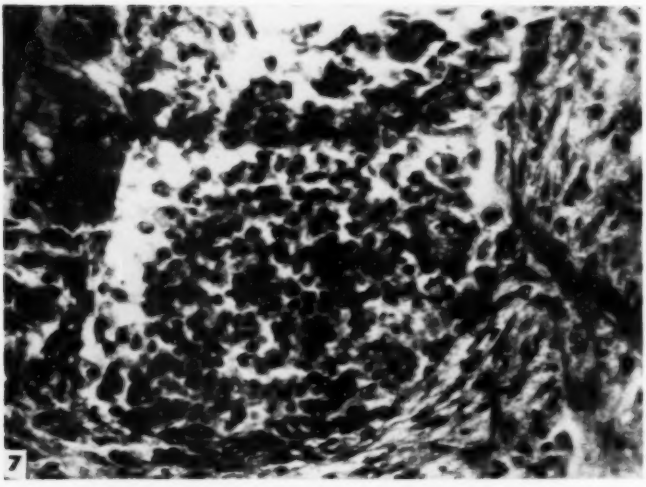

FIG. 7 (Case II): The leucocytes form purulent foci.

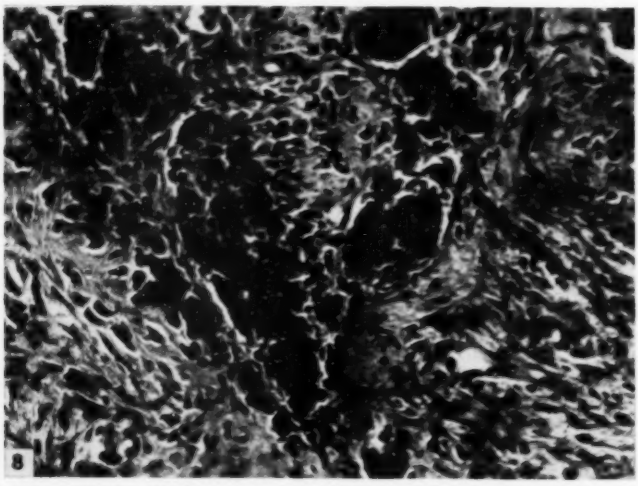

FIG. 8 (Case II): In the fibrous stroma much osteoid is being laid down and this is undergoing calcification in an irregular manner. There are osteoclasts around these foci of calcification, but they do not show well.

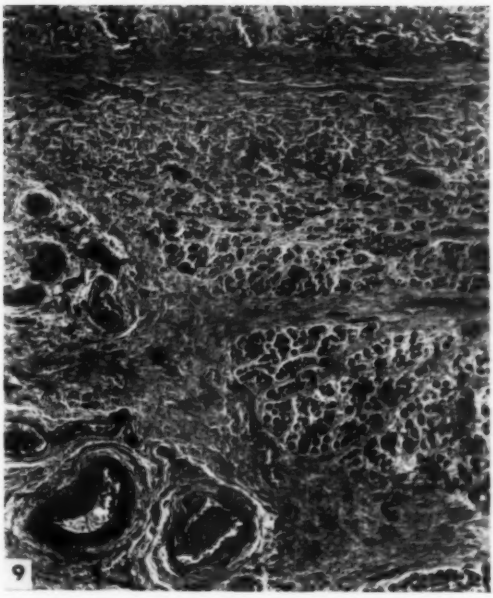

FIG. 9 (Case II): The soft tissues and muscle adjacent to the bone show now much scar tissue which separates the muscle fibers. Muscle in the scar tissue is undergoing atrophy. The blood vessels are normal.

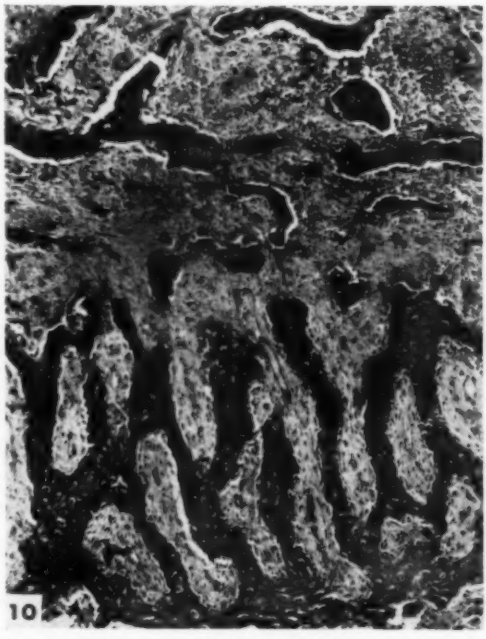

FIG. 10 (Case II): Forty days after the initial biopsy the second biopsy shows the callous-like process has been replaced by a cap of well organized medullary and cortical bone. The marrow spaces are filled with connective tissue and the inflammatory cells have mostly disappeared, there being only a few lymphocytes present.

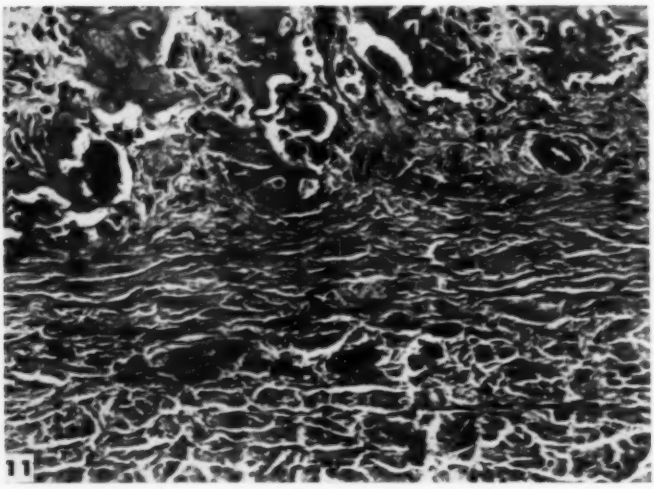

FIG. 11 (Case II): The periosteum is composed of hyaline scar tissue. A bony cortex is forming. A few osteoclasts are present, but do not show well.

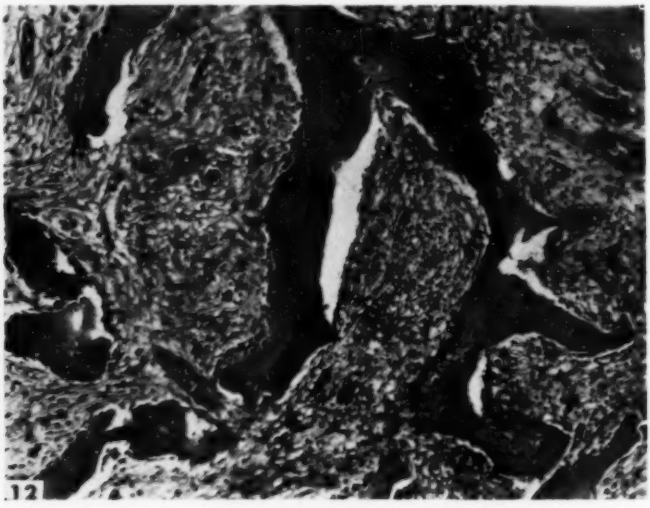

FIG. 12 (Case IV): A cellular connective tissue fills the marrow spaces.

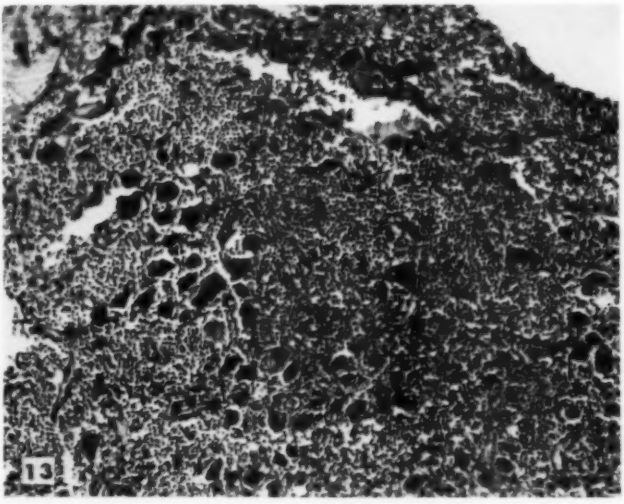

FIG. 13 (Case IV): Purulent foci containing numerous osteoclasts are shown. In such foci the bony trabeculae are destroyed and this, in all probability, produced the "cysts" seen in the x-ray.

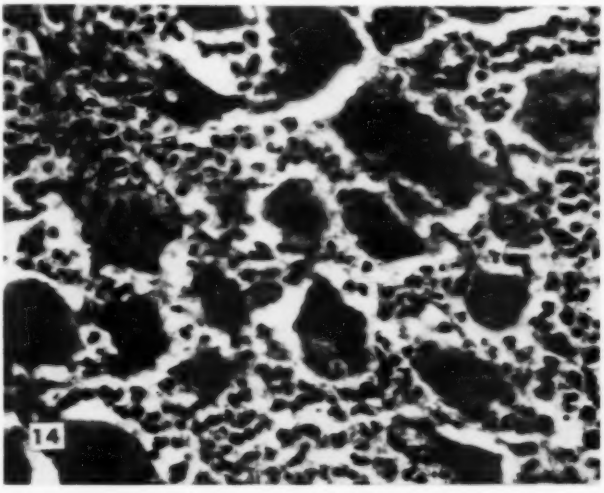

FIG. 14 (Case IV): High power shows the numerous osteoclasts and polymorphonuclear leucocytes.

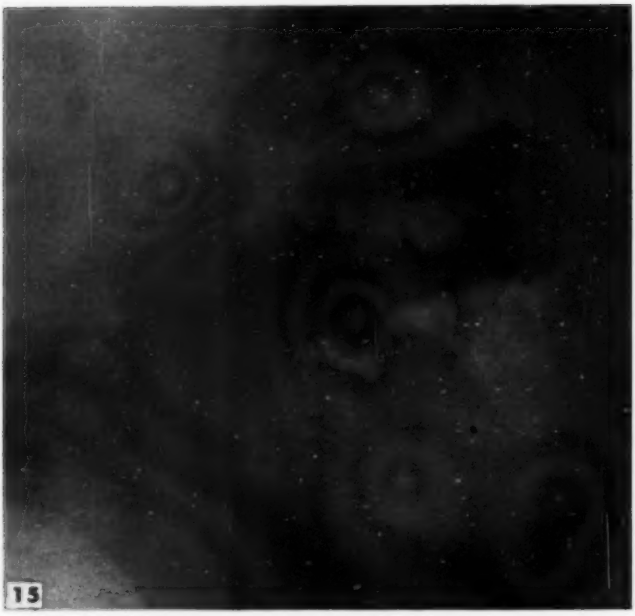

FIG. 15 (Case IV): The x-ray of Case IV shows ill-defined cysts in the greatly enlarged mandible.

FIG. 16 (Case V): A cap of cortical and medullary bone has formed over the original bone. The marrow spaces are filled with a fibrous tissue stroma. The bony trabeculae are more numerous than normal.

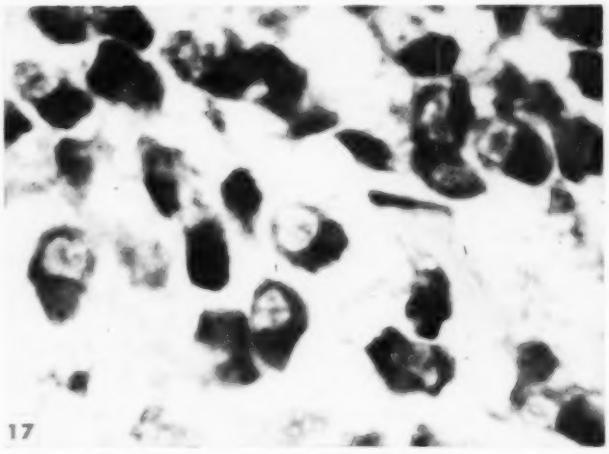

FIG. 17 (Case V): High power magnification shows the vacuoles in osteoblasts. These contain ill-defined reticular net-works.

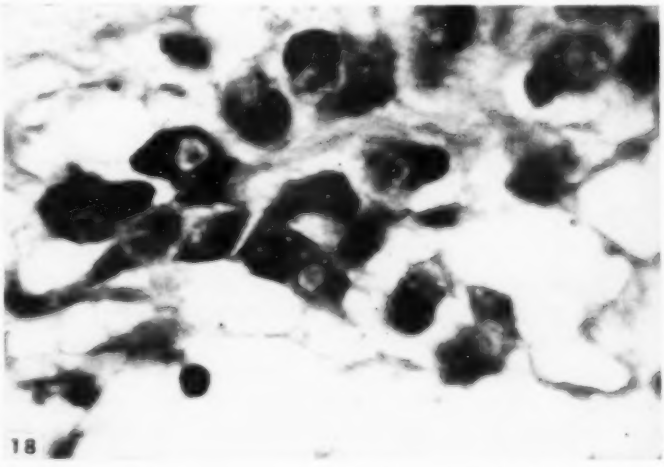

FIG. 18 (Case VI): High power magnification shows vacuolated osteoblasts similar to Figure 17.

AN ASSAY OF THE SKELETOGENIC EFFECT OF L-TRIIODOTHYRONINE AND ITS ACETIC ACID ANALOGUE IN IMMATURE RATS

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The finding, announced by Gross and Pitt-Rivers (1), that 3,5,3′-L-triiodothyronine is at least five times as potent as L-thyroxine has spawned hundreds of new studies on the analogues of thyroxine. Pitt-Rivers (2) has reported that, "Tetraiodothyroacetic and triiodothyroacetic acid both possess about one-tenth of the activity of L-triiodothyronine . . . Triiodothyroacetic acid has been shown to have considerable activity in measuring the metabolic rate of rats." The likelihood that one of these acetic acid analogues (triiodothyroacetic acid in particular) represents the ultimate physiologically active derivative of the thyroid hormone is based upon the findings that these compounds act without a latency period (3, 4), that triiodothyroacetic acid is detectable in the kidneys of triiodothyronine-treated rats (5), and by the fact that "the conversion of thyroxine and triiodothyronine to their respective acetic acid analogues *in vitro* by an enzyme prepared from rat kidney mitochondria" has been accomplished (6).

The biological procedures currently in vogue for evaluating the relative activity of thyroxine derivatives are based upon one of the following effects:

1) acceleration of oxygen consumption in rats or mice,
2) inhibition of thiouracil-induced goitre formation in rats,
3) promotion of metamorphosis in tadpoles.

For bioassay purposes these are highly sensitive test objects. However, it was felt that an assay based upon the rate of skeletal differentiation in the rat would provide valuable supplementary data. Promotion of skeletal maturation is a specific, cumulative and irreversible effect of thyroxine, which is demonstrable histologically and represents a direct peripheral action of the hormone. In normal rats all of the ossification centers of the carpus, tarsus and caudal vertebrae as well as the epiphyseal centers of the extremities are established during the first three weeks after birth (7). The time of appearance of each of these centers is so characteristic that skeletal age estimates of normal immature rats may be made with an accuracy of one-half day.

The purpose of this report is to describe a bioassay procedure based upon skeletal maturation. In addition, using this method, the results obtained from the assay of L-thyroxine, L-triiodothyronine and their respective acetic acid analogues are presented. Dr. R. Pitt-Rivers very generously provided the acetic acid derivatives used in the present analysis.

TABLE I

Dosage Scale of L-Triiodothyronine and L-Thyroxine and the Number of Litters Treated

Number of Litters per Dosage Level	Daily Dosage per 10 Grams of Body Weight		
	L-Triiodothyronine microgm.	L-Thyroxine microgm.	Molar equivalent
2	0.18	0.25	0.3×10^{-9}
4	0.38	0.50	0.6×10^{-9}
8	0.75	1.00	1.1×10^{-9}
5	1.20	1.60	1.8×10^{-9}
3	1.50	2.00	2.3×10^{-9}

MATERIALS AND METHODS

Twenty-two newborn litters each consisting of three male and three female albino rats were used to assay L-thyroxine and L-triiodothyronine at five different dosage levels, as shown in Table I. One male and one female of each litter were treated with L-triiodothyronine, another pair of opposite sex received equimolar amounts of L-thyroxine,[1] while the third pair served as uninjected controls.

Three all-male litters and three all-female litters, a total of 36 animals, were used to assay the acetic acid analogues. One animal of each litter received triiodothyroacetic acid, another received tetraiodothyroacetic acid and, in order to provide a sound basis for comparison, a third littermate received L-triiodothyronine, a fourth L-thyroxine, while the remaining pair served as uninjected controls. A single dosage level of 1.8×10^{-9} moles per 10 gms. body weight per day was selected since, as established beforehand, this represents the maximum dosage of L-triiodothyronine that is well tolerated by infant rats.

Injections were administered b.i.d., half of the daily dosage given in the morning, half in the evening. Each injection was aseptically administered by the intraperitoneal route, in a volume of 0.05 ml. per 10 gms. of body weight, by means of a tuberculin syringe fitted with a 30 gauge needle. To avoid leakage of the injection from the peritoneal cavity it was necessary to "thread" the hypodermic needle tangentially through the abdominal wall of the infantile rats. The period of treatment varied from five to twenty-one days, although the majority of animals were autopsied at eight, eleven or sixteen days of age. The body weight of each animal was measured daily and the ages noted at which the incisors erupted and the eyelids became separated.

Autopsy was performed approximately twelve hours after the last injection. The animals were killed by an overdose of sodium pentobarbital, eviscerated, and stripped of pellage, muscle and adipose connective tissue as extensively as was deemed feasible without risking injury to the skeleton. Following dissection the skeletal specimens were stored in absolute ethyl alcohol for two to four days; thereafter, they were cleared by Mall's (8) method and stained by Dawson's (9) method. These procedures call for maceration in a 1 per cent to 2 per cent aqueous solution of potassium hydroxide for two to four days, and staining for one to three days in an aqueous solution consisting of 0.1 gm. of alizarin red in one liter of 0.5 per cent potassium hydroxide. Final clearing was achieved by transferring specimens through a series of glycerine solutions of increasing strength, specifically, one to three days

[1] Through the courtesy of Dr. D. E. Holtkamp the preparations of L-triiodothyronine and L-thyroxine were provided by the Smith, Kline and French Laboratories, Philadelphia 1, Pennsylvania.

treatment in each of 20 per cent, 40 per cent, 60 per cent, 80 per cent and 100 per cent glycerine solutions. From rats twenty-one days of age at the time of autopsy, the humerus was excised bilaterally and fixed in 10 per cent formol. Only the distal half of the humerus was prepared as follows for histologic examination: decalcificated in 5 per cent HNO_3, dehydrated in alcohol, cleared in oil of cloves, embedded in tissue-mat, sectioned in the frontal plane at 8 microns and stained with hematoxylin and eosin or Heidenhain's azan.

A thorough examination was made of each of the alizarin-stained, cleared skeletal specimens. In particular, the most recently established centers of calcification were sought since it is upon the presence of these that the "skeletal" age estimates were based. The calcification centers used in these assays are listed in the chronological order of their appearance in Table II. These data were obtained from normal control specimens and may be used to make skeletal age estimates from seven to twenty-seven days, accurate to within one-half day. However, it must be emphasized that in comparative studies based upon skeletal age determinations the normal controls should be of the same litter as the experimental rats. A photographic atlas has been prepared to show all ossification centers in the rat as they first appear in the normal sequence of development (10). Criteria for estimating skeletal ages between fifteen and forty days have been set forth by Becks *et al.* (11), based upon histologic examination of the distal end of the humerus. Advantage was taken of these histological criteria to supplement the information obtained from macroscopic examination of skeletal specimens in the case of rats twenty-one days of age.

RESULTS

Effects of L-Triiodothyronine and L-Thyroxine

Skeletal maturation: The most pronounced acceleration of skeletal maturation was achieved with L-triiodothyronine, which doubled the rate of skeletal differentiation when given at a dose level of 1.8×10^{-9} moles per 10 gms. of body weight per day (Fig. 1). Thus, at a chronologic age of eight days, these experimental animals had attained a status of skeletal differentiation equivalent to that of normal eighteen-day-old rats. Eight days' treatment with an equimolar amount of L-thyroxine advanced the skeletal age by five to six days (Fig. 1). However, with continued treatment a gradual deceleration of skeletal maturation rate was observed so that, even by three weeks of age, the skeletal age of L-triiodothyronine-treated rats exceeded that of the untreated littermates by only nine days, and exceeded that of the L-thyroxine-treated littermates by about four days. The status of skeletal maturation of rats autopsied at twenty-one days of age, was determined through study of the histologic sections of the distal end of the humerus as well as from macroscopic examination of the remainder of the skeleton. Photomicrographs of the distal end of the humerus of rats representative of their group are shown in Figures 4, 5, and 6. Normally at twenty-one days of age the epiphyseal plate of the humerus (Fig. 4) is a thick cartilaginous plate, imperforate or interrupted only where it abuts against the medial epicondyle. The latter consists largely of calcified cartilage with at most a minute osseous center. In rats treated from birth until twenty-one days of age with L-triiodothyronine at the 1.8×10^{-9}

TABLE II

Times of Appearance of Calcification Centers in Rats 7 to 27 Days of Age

Skeletal Part	New Center of Calcification	Days
Carpus	Pisiform and scapholunate	7
Forepaw, digit I	Diaphysis of middle phalanx	7
Hyoid	Greater horns	7
Radius	Distal epiphysis	7
Vertebrae, caudal	Centra, nos. 21, 22, 23	7
Carpus	Centrale and triquetrum	8
Femur	Distal epiphysis	8
Forepaw, digit I	Diaphysis of proximal phalanx	8
Metacarpals (all)	Epiphyses	8
Radius	Proximal epiphysis	8
Tarsus	Cuboid	8
Tibia	Proximal and distal epiphyses	8
Ulna	Distal epiphysis	8
Vertebrae, caudal	Centra, nos. 24, 25, 26	8
Carpus	Capitate	9
Humerus	Proximal epiphysis	9
Ulna	Proximal epiphysis	9
Tarsus	Medial cuneiform	10
Carpus	Trapezium	11
Carpus	Trapezoid	12
Fibula	Distal epiphysis	12
Tarsus	Lateral cuneiform	12
Forepaw, digits (all)	Epiphyses of proximal phalanges	13
Metatarsals (all)	Epiphyses	14
Tarsus	Epiphysis of calcaneus	14
Carpus	Falciform and epiphysis of pisiform	15
Forepaw, digits (all)	Epiphyses of middle phalanges	15
Humerus	Medial epicondyle	15
Tarsus	Intermed. cuneiform and navicular	15
Hindpaw, digits (all)	Epiphyses of proximal phalanges	16
Metacarpo-phalangeal joints	Sesamoids, one pair on volar aspect of each joint	17
Vertebrae, caudal	Epiphyses, nos. 1, 2, 3	17
Femur	Lesser trochanter	18
Tarsus	Tibiale	18
Vertebrae, caudal	Epiphyses, nos. 4, 5	18

TABLE II—*Continued*

Skeletal Part	New Center of Calcification	Days
Hindpaw, digits (all)	Epiphyses of middle phalanges	19
Vertebrae, caudal	Epiphyses, nos. 6, 7	19
Femur	Greater trochanter	20
Vertebrae, caudal	Epiphyses, nos. 8, 9	20
Hyoid	Lesser horns	21
Vertebrae, caudal	Epiphyses, nos. 10, 11	21
Fibula	Proximal epiphysis	22
Knee joint	Patella	22
Vertebrae, caudal	Epiphyses, nos. 12, 13	22
Metatarso-phalangeal joints	Sesamoids, one pair on volar aspect of each joint	23
Vertebrae, caudal	Epiphyses, nos. 14, 15	23
Femur	Proximal epiphysis (head)	24
Hindpaw, distal interphalangeal joints	Sesamoids, one on volar aspect of each joint	24
Knee joint	Fabellae, medial and lateral	24
Vertebrae, caudal	Epiphyses, nos. 16, 17	24
Forepaw, distal interphalangeal joints	Sesamoids, one on volar aspect of each joint	25
Knee joint	Semilunar articular cartilages	25
Vertebrae, caudal	Epiphyses, nos. 18, 19	25
Tibia	Tuberosity	26
Vertebrae, caudal	Epiphyses, nos. 20, 21, 22, 23	26
Acetabulum	Epiphysis of iliac component	27

molar level, the main epiphyseal plate of the distal end of the humerus is represented only by two thin cartilaginous remnants, one in the lateral epicondyle, another midway between the epicondyles (Fig. 5). The ossification center of the medial epicondyle is fully expanded, having replaced its cartilaginous precursor. Thus, as demonstrated in the humerus, L-triiodothyronine hastened the closure of an epiphyseal plate as well as the onset of appearance of secondary centers of ossification. In the case of the L-thyroxine-treated littermates the main epiphyseal plate of the distal end of the humerus was perforated at one or two points, elsewhere the disc was of substantial thickness with no indication that complete resorption of the plate was imminent. A small osseous center is seen in the medial epicondyle (Fig. 6).

At a still higher dosage level (2.3×10^{-9} moles per 10 gms. of body weight) both L-triiodothyronine and L-thyroxine were so toxic that the majority of

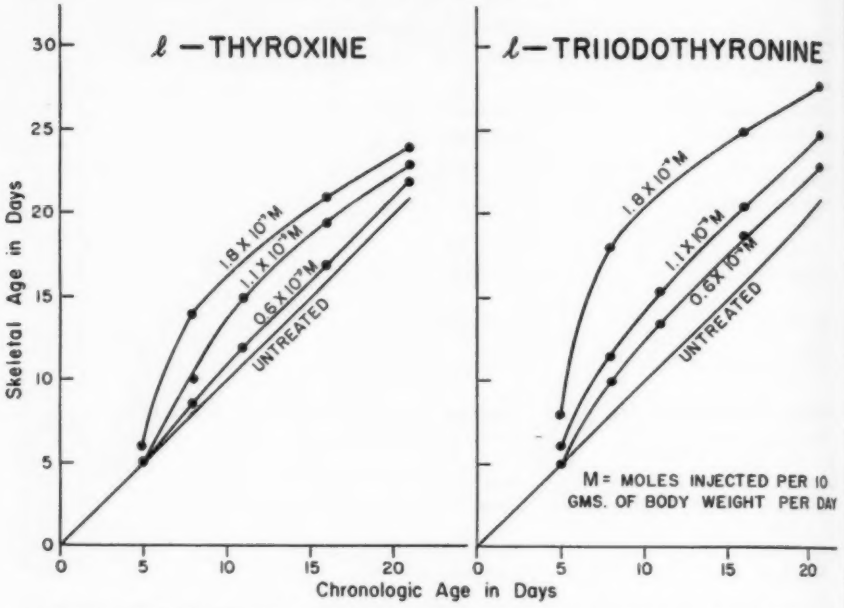

FIG. 1. Skeletal maturation curves of experimental rats as treated at three different dosage levels with L-thyroxine or L-triiodothyronine.

TABLE III

Effect of L-Triiodothyronine and L-Thyroxine on Ages at which Eyelids Separate and Lower Incisors Erupt

Developmental Event	Daily Dosage in Mols per 10 Gms. B. W.	Untreated Controls	L-Triiodothyronine	L-Thyroxine
		Days of age	Days of age	Days of age
Separation of Eyelids	0.3×10^{-9}	15	13½	14½
	0.6×10^{-9}	15	12½	14
	1.1×10^{-9}	14½	11	12½
	1.8×10^{-9}	14½	10	11½
Initial Eruption of Lower Incisors	0.3×10^{-9}	9½	8	8½
	0.6×10^{-9}	9	7	7½
	1.1×10^{-9}	9	6	7
	1.8×10^{-9}	9	5½	6½
	2.3×10^{-9}	9	5	5½

animals so treated did not survive beyond eight days of age. A larger number of animals, eight litters, were treated at the comparatively moderate dose level of 1.1×10^{-9} moles per 10 gms. of body weight per day. By one week of age the L-triiodothyronine-injected rats had attained a skeletal age of 10 days and, throughout the second and third weeks of treatment, skeletal differ-

entiation was maintained at a margin of about 4 days in advance of that of the normal untreated littermates, but only 1 day in advance of that of their L-thyroxine-injected littermates. The lower dosage levels of 0.6×10^{-9} moles and 0.3×10^{-9} moles of triiodothyronine per 10 gms. of body weight per day maintained skeletal maturation 2½ and 1½ days in advance of the controls, respectively. Thyroxine at the equimolar dose levels maintained an advance in skeletal age of 1 day and ½ day, respectively.

Eruption of the incisors and separation of the eyelids: In normal rats the gums are first perforated by the incisors about 9½ days after birth, and the eyelids become separated from one another on the 15th day after birth. The hastening effect of the thyroid hormones upon these developmental events is summarized in Table III. Triiodothyronine in a dose of 1.8×10^{-9} moles per 10 gms. of body weight effected eruption of the incisors 4 days prematurely and parting of the eyelids 5 days prematurely. Thyroxine, administered at an equimolar dose level, caused these events to occur 3½ and 4 days earlier than normal, respectively. At lower dosage levels, L-triiodothyronine consistently accelerated these developmental events somewhat more than an equimolar dose of L-thyroxine.

Growth: Body weight gain was significantly depressed by L-triiodothyronine when administered at the 1.8×10^{-9} molar level (Fig. 3). On the other hand, at an equimolar level, L-thyroxine caused little or no retardation of body weight gain.

Effects of the acetic acid analogues

Skeletal maturation: Triiodothyroacetic acid was found to be a potent skeletal maturation-promoting agent. By 8 days of age, the triiodothyroacetic acid-treated rats had achieved a status of skeletal differentiation nearly equivalent to that of the untreated 16-day-old controls (Figs. 8 and 10). This pronounced acceleration in rate of skeletal differentiation was not sustained. By the 16th day of treatment the skeletal age of rats receiving triiodothyroacetic acid exceeded that of their littermate controls by 6 days, and by the 21st day of treatment this margin of advance was only 4 days. Thus, the "bone age curve" of the triiodothyroacetic acid-treated animals was intermediate between those of the L-thyroxine- and L-triiodothyronine-injected littermates (Fig. 2).

On the other hand, tetraiodothyroacetic acid was decidedly less potent. A 3 days' advance in skeletal age, achieved after 8 days of injection, was about the maximum extent to which skeletal differentiation could be accelerated by tetraiodothyroacetic acid (Fig. 9). Only 1 day's advance after 16 days of treatment, and no advance over the controls was detectable at 21 days of age (Fig. 2).

Eruption of the incisors and separation of the eyelids: Triiodothyroacetic acid effected eruption of the incisors at 6½ days of age and separation of the eye-

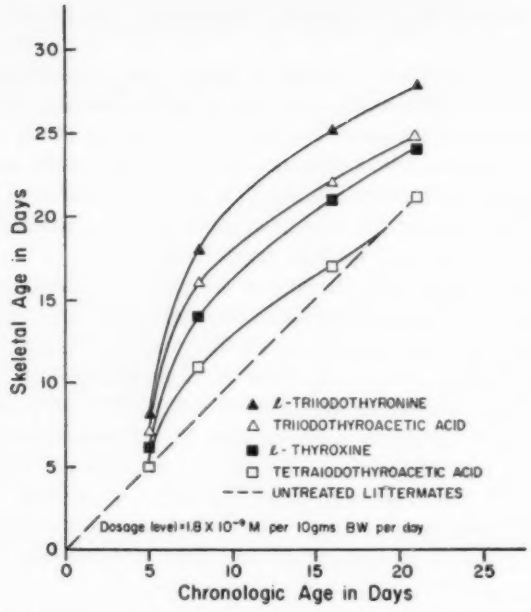

FIG. 2. Skeletal maturation curves of experimental rats plotted against the normal base-line.

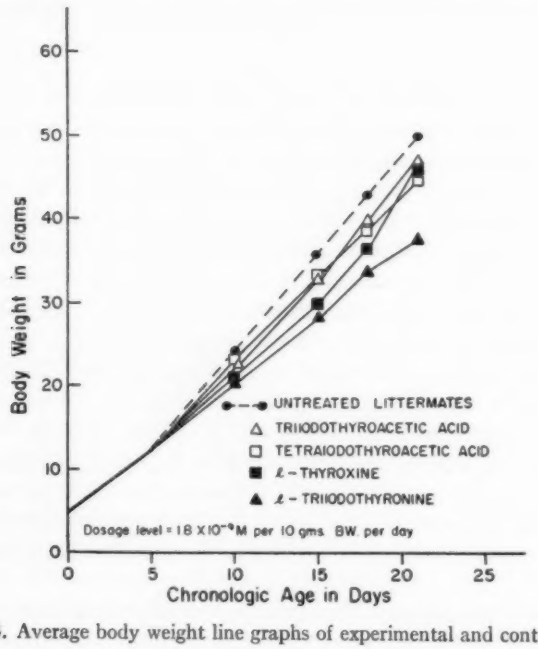

FIG. 3. Average body weight line graphs of experimental and control rats.

lids at 11 days, hence, 3 and 4 days prematurely, respectively. Tetraiodothyroacetic acid caused no departure from the normal with regard to the time of occurrence of these events.

Growth: Neither of the acetic acid analogues produced any alteration in rate of body weight gain (Fig. 3).

DISCUSSION

Within the limits imposed by differences of technic the data of the present study are in agreement with those of Hoskins (12), Noback, Barnett and Kupperman (13), Tusques (14), and Morton and Fahn (15), all of whom have measured the hastening effect of thyroxine on the development of unoperated, newly born rats. In the previous studies, however, criteria for making skeletal age estimates were not presented, nor was there any attempt to establish dose-effect curves. Tusques (16) compared the effect of L-triiodothyronine and L-thyroxine on the times of eyelid-opening, pinnae-erection and transformation of the pellage, and stated that 25 microgm. of L-triiodothyronine was of a potency equivalent to 75 microgm. of L-thyroxine. The incisor eruption rate in adult rats has also been used to assay thyroxine (17) and triiodothyronine (18). Fell and Mellanby have assayed the effect of L-thyroxine (19) and L-triiodothyronine (20) on explanted limb-bone rudiments. These studies are particularly interesting as they demonstrate direct histologic effects produced by minute dosages of the hormones. Up to the present time no reports have appeared concerning the effect of the acetic acid analogues on skeletal maturation.

SUMMARY

A method for assaying skeletal maturation-promoting agents has been presented. By this method L-thyroxine, L-triiodothyronine and their respective acetic acid analogues were assayed. The most potent of these, L-triiodothyronine, at a daily dosage level of 1.8×10^{-9} moles per 10 gm. of body weight, more than doubled the rate of skeletal differentiation during the first eight days' treatment. Within the dose ranges studied the skeletogenic effect of L-triiodothyronine approximated that of twice the equimolar dose of L-thyroxine. The skeletogenic activity of triiodothyroacetic acid was slightly greater than that of L-thyroxine, whereas the activity of tetraiodothyroacetic acid was only one-half that of L-thyroxine. As reflected by the times of incisor eruption and eyelid opening, the degree of developmental acceleration was consistent in each case with the skeletogenic effects of the analogues studied. Only L-triiodothyronine caused a significant depression in rate of body weight gain.

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EXPLANATION OF ILLUSTRATIONS

Photomicrographs of distal end of humerus of rats 21 days of age. Frontal sections, hematoxylin and eosin. Reduced ⅔ from mag. ×20.

FIG. 4. Normal.

FIG. 5. Injected with 1.8×10^{-9} moles of L-triiodothyronine per 10 gm. of body weight per day from birth. Using the criteria of Becks *et al.*, the skeletal age of this specimen was estimated to be twenty-eight to thirty days.

FIG. 6. Injected with 1.8×10^{-9} moles of L-thyroxine per 10 gm. of body weight per day from birth. The skeletal age was estimated to be twenty-four to twenty-five days.

The following photographs are of alizarin-stained specimens of the right hindpaw of rats as seen from the dorsal aspect. All of the specimens except that shown in Fig. 10 are from eight-day-old littermates. ×5.

FIG. 7. Normal, age eight days. The smallest dark dot representing the most recently acquired calcification center is of the cuboid.

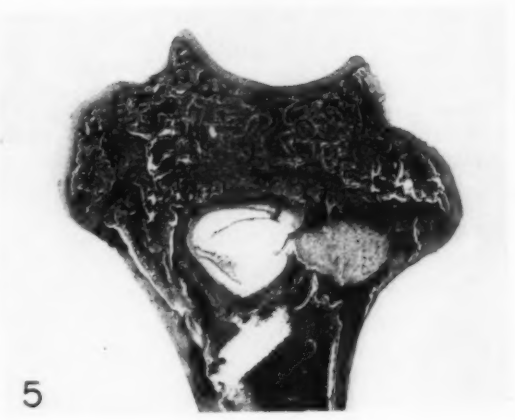
FIG. 8. Triiodothyroacetic acid treated. All of the tarsal centers except that of the tibiale are present and nearly of definitive size. All four metatarsal epiphyses are well developed but the epiphysis of the proximal phalanx of digit III must have been established only a few hours prior to autopsy. Using the data of Table II the skeletal age of this specimen was estimated to be fifteen-and-one half to sixteen days.

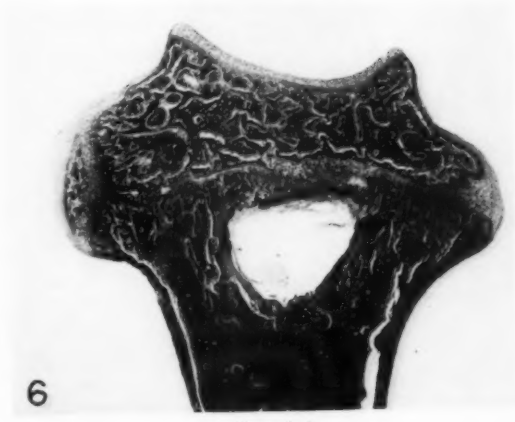
FIG. 9. Tetraiodothyroacetic acid treated. Of three smaller tarsal centers seen that of the lateral cuneiform was most recently established. The distal epiphyseal center of the fibula has not appeared. The skeletal age of this specimen was estimated to be eleven-and-one-half days.

FIG. 10. Normal, age sixteen days. The epiphyseal centers of the proximal phalanges have been established within one-half day.

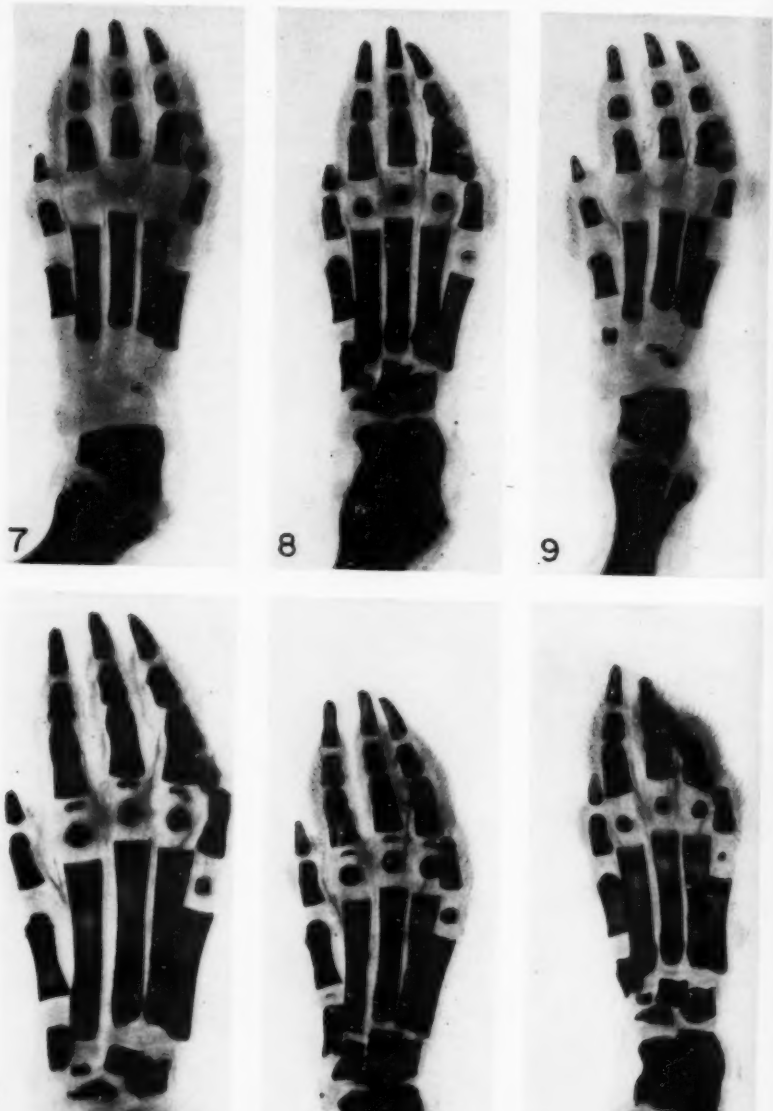
FIG. 11. L-Triiodothyronine treated. The minute center of the tibiale was established only a few hours prior to autopsy. The skeletal age of this specimen was estimated to be eighteen days.

FIG. 12. L-Thyroxine treated. The skeletal age was estimated to be fifteen days.





FIGS. 4-6



FIGS. 7–12

ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY IN ASSOCIATION WITH THE ALPHA OMEGA ALPHA FRATERNITY

May 20, 1957

THE INHERITANCE OF AN INTRINSIC ABNORMALITY OF THE RED BLOOD CELL PREDISPOSING TO DRUG INDUCED HEMOLYTIC ANEMIA

EUGENE A. BROWNE

The administration of certain drugs such as primaquine, sulfonilamide, acetanilid and nitrofurantoin results in the development of an acute hemolytic anemia in some individuals. It has been demonstrated that this type of drug reaction is associated with an intrinsic abnormality of the red blood cell, a defect known to be rare among Caucasians but occurring in about 11 per cent of a group of 199 American Negroes given primaquine.

The abnormality can be detected *in vitro* by determining the reduced glutathione (GSH) stability of the erythrocytes upon incubation with acetylphenylhydrazine; the GSH of drug-sensitive cells is unstable under these conditions whereas that of nonsensitive cells is stable.

A study of the incidence and mode of inheritance of drug sensitive red cells was undertaken in the Departments of Medicine and Pediatrics of The Johns Hopkins Hospital by application of this method.

A random group of 131 Negro males and 165 Negro females was examined for the presence of this abnormality. Twenty of the males or 15 per cent were found to be sensitive individuals. Only 3 females or approximately 2 per cent were found to be unequivocally drug-sensitive. There were in addition many females whose GSH levels ranged between those of the clearly sensitive and the nonsensitive subjects. The incidence of intermediate values was found to be much higher in the female kindred of drug-sensitive individuals than in the random group of 165 Negro females.

Seventeen families of known drug-sensitive individuals consisting of 31 sibships were studied. Only 12 per cent of the fathers of sensitive sons had the red cell defect whereas 72 per cent of the mothers had either unequivocally sensitive erythrocytes or intermediate red cell GSH values.

Women whose erythrocytes were definitely drug sensitive had a total of 9 sons. All 9 were definitely drug-sensitive. The fathers of these women, whenever tested, were shown to be drug-sensitive. The abnormality was observed to skip a generation in several affected families.

Drug sensitive red cells appeared to be transmitted by a sex linked gene of intermediate dominance. Males who were homozygous and females who were homozygous for the abnormal gene were postulated to be drug-sensitive. Furthermore, females heterozygous for this gene may show either intermediate or normal erythrocyte GSH levels. The incidence of Negro females heterozygous for this gene was calculated to be about 26 per cent. The observed incidence of 2 per cent for Negro females with drug sensitive red cells agreed well with the expected incidence of female homozygotes of 2 per cent. This further supported the hypothesis that we were dealing with a sex linked gene of intermediate dominance.

SOME BIOCHEMICAL PROPERTIES OF THYROXINE

KARL A. SCHELLENBERG[1]

Many recent studies of the action of thyroxine have demonstrated diverse biochemical effects. Thyroxine has been found to effect a swelling of mitochondria *in vitro*, to combine with heavy metals, and to act as a competitive inhibitor for the kidney enzyme d-amino acid oxidase.

Another enzyme even more significant for amino acid oxidation, and of central importance in protein metabolism, is liver l-glutamic dehydrogenase (GAD). The reaction of GAD is schematically:

$$\text{l-glutamate} + H_2O + DPN^+ \underset{\longleftarrow}{\xrightarrow{GAD}} \alpha\text{-ketoglutarate} + DPNH + NH_4^+$$

Caughey, Smiley, and Hellerman have found that substances possessing two anionic centers 7.5 Angstroms distant, similarly to glutamate, are competitive inhibitors for glutamate in the forward reaction. Thyroxine and its analogs bear a formal resemblance to the competitive inhibitors, but are found to inhibit uncompetitively with respect to glutamate and DPN at a concentration of 10^{-6} to 10^{-5} molar.

The action of thyroxine on GAD was further investigated by us in order to throw light on enzyme mechanism and possible control of activity by hormones. More generally this emphasizes the mode of combination of thyroxine with substances such as proteins. The inhibition is reversible on dilution and also on addition of cysteine or glutathione. This prompted a more detailed study of the enzyme sulfhydryl groups in order to determine whether thyroxine possibly controls enzyme activity through alteration of thiol groups.

Titration of the crystalline enzyme with heavy metals (silver ion, phenylmercury, methylmercury ions) revealed that the protein possesses per 28,000 grams one sulfhydryl group apparently nonessential for activity and a second sulfhydryl the combination with which alters the activity profoundly. The native enzyme exhibits a nitroprusside reaction characteristic of thiols which was abolished on combination with the heavy metals or other sulfhydryl-characterizing agents but not by thyroxine.

No evidence was found for combination of thyroxine or an air-oxidized derivative with enzyme sulfhydryl groups. It was concluded that thyroxine alteration of enzyme activity though "antagonized" by thiol compounds, is not mediated through combination of thyroxine with sulfhydryl anions. A sidelight of the investigation was the discovery of rather remarkable structure-activity relations of the heavy metals. Under identical conditions silver abolished enzyme activity, phenylmercury had no effect, and methylmercury increased enzyme activity two-fold.

THE PATHOGENESIS OF FEVER: EVIDENCE FOR DIRECT AND INDIRECT ACTION OF BACTERIAL ENDOTOXIN

WILLIS R. KEENE

ENZYMATIC SYNTHESIS OF ASCORBIC ACID IN ANIMAL TISSUES

ARTHUR P. GROLLMAN

The biosynthetic pathway of D-glucuronic acid of L-ascorbic acid has been investigated in various animal species. Particular reference has been made to the guinea pig, monkey and

[1] Henry Strong Denison Scholar 1955–1957.

man as these are the only higher species requiring the vitamin in their diet. The intermediates are shown in the following reaction sequence:

$$\text{D-glucuronate} \rightleftharpoons \text{L-gulonate} \longrightarrow \text{[3-keto-L-gulonate]} \begin{matrix} \nearrow \text{L-ascorbate} \\ \rightleftharpoons \text{L-xylulose} + CO_2 \end{matrix}$$

We have studied the individual steps in detail. The enzyme catalyzing the reduction of D-glucuronate to L-gulonate has been found to be present in the livers and kidneys of those species studied including man, monkey, guinea pig, cow, pig, dog, rat and pigeon. A partially purified enzyme from pig kidney was used in studying this and other reactions. The identity of glucuronate and gulonate was established by paper chromatographic and isotopic procedures. Triphosphopyridine nucleotide was found to be the specific cofactor required in this step.

A second enzyme, which oxidizes gulonate, is found in liver and kidney extracts of all species tested. It requires diphosphopyridine nucleotide as its specific cofactor. A reaction product has been identified as L-xylulose which presumably arises from the decarboxylation of 3-keto-L-gulonate. It was found, moreover, that appropriate alternations in the experimental conditions resulted in the accumulation of ascorbic acid from L-gulonate; presumably 3-keto-L-gulonate was the immediate precursor. Therefore, the keto sugar acid is an intermediate in two divergent pathways, one of which leads to the formation of the vitamin. This pattern was also found to occur in tissues of primates and guinea pigs. However, in these species, the reaction leading to the formation of L-xylulose is followed to the complete exclusion of that forming ascorbic acid and the vitamin must necessarily be provided in the diet.

The genetic defect in this sequence of enzymatic reactions may be expressed, not primarily as the lack of an enzyme, but possibly in terms of the relative activities of two enzymes competing for the same intermediate.

THE EFFECT OF CERTAIN ANTERIOR PITUITARY HORMONE PREPARATIONS ON FORELIMB REGENERATION IN THE HYPOPHYSECTOMIZED NEWT TRITURUS VIRIDESCENS VIRIDESCENS

JAMES A. WILKERSON

Since it has been previously shown that regeneration of the forelimb will not occur in hypophysectomized newts and that injections of an anterior pituitary extract will restore this ability, this study was undertaken in an effort to discover more specifically which hormones were responsible for these phenomena.

Somatotrophin, a commercial preparation available from Armour and Co. containing 80 per cent somatotrophic hormone, 20 per cent thyrotrophic hormone, a trace of gonadotrophic hormone, and no adrenocroticotrophic hormone, was injected into thirty-four hypophysectomized newts for fifteen days immediately following amputation of the forelimb. All thirty-four of these animals were regenerating their limbs at the time of death from thirty to seventy days later. Histological examination revealed the experimental limbs to be similar to normal control limbs except for a thickened epithelium on the outside of the limb.

To test the hypothesis that by fifteen days after amputation, if no hormone or other therapy is given, scar tissue will have grown over the limb stump in the hypophysectomized animal preventing regeneration, seven animals were given the somatotrophin preparation described above for fifteen days beginning fifteen days after amputation. All of these animals regenerated their limbs also.

Control animals which were given a preparation containing only thyrotrophic hormone,

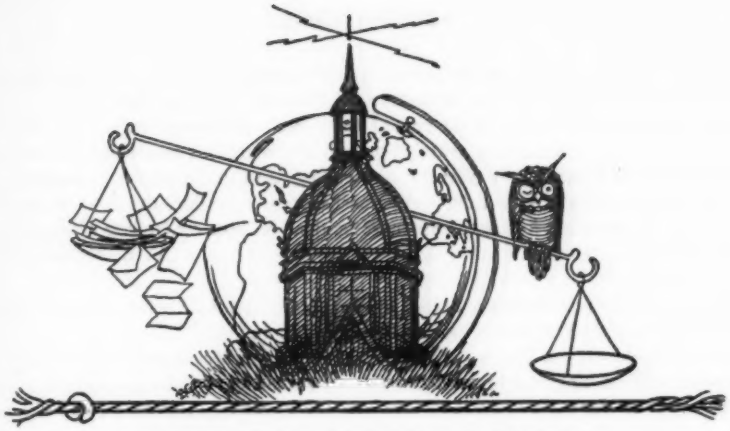
also available from Armour and Co., showed regeneration in only one out of fifteen animals. The other fourteen animals showed no signs of regeneration.

It was concluded that the somatotrophin preparation which was used contained the hormones essential to regeneration of the forelimb in the newt, Triturus viridescens viridescens, and that thyrotrophin alone would not support regeneration.

THE MECHANISM OF HEMOLYTIC ANEMIA INDUCED BY NITROFURANTOIN (FURADANTIN)

EXALL L. KIMBRO, JR.

Two Negro patients were observed to develop hemolytic anemia while receiving nitrofurantoin for treatment of urinary tract infections. One of these patients was available for further study. It was found that his erythrocytes contained less than the normal concentration of reduced glutathione (GSH). Upon incubation of his blood with acetylphenylhydrazine, there was a precipitous fall in erythrocyte GSH content. These changes are like those shown by Beutler *et al.* to be characteristic of the intrinsic red cell defect of those individuals uniquely susceptible to the hemolytic action of certain drugs such as primaquine. Further study demonstrated a striking fall in erythrocyte GSH upon incubation of 1.0 ml. of the patient's whole blood with 0.1 mg. nitrofurantoin, whereas no essential change was observed in the GSH of normal cells with as much as 1.0 mg. per ml. Blood was obtained from 4 other Negro subjects whose erythrocytes had previously been determined to be of the primaquine-sensitive type by incubation with acetylphenylhydrazine. A marked reduction of erythrocyte GSH was observed in each of the four when 1 ml. of blood was incubated with 0.1 mg. nitrofurantoin. It thus seems probable that nitrofurantoin may induce hemolytic anemia in the same subjects who could be expected to have a hemolytic reaction during administration of primaquine, and by the same mechanism. In a random survey of 296 Negroes, 23 primaquine-sensitive subjects were found, an incidence of 7.8 per cent.



BOOK REVIEWS

The Compleat Pediatrician, 7th edition. By W. C. DAVISON AND JEANA DAVISON LEVINTHAL. 272 pp., $4.25. Duke University Press, Durham, N. C.

The new edition of this classic in reference manuals is very welcome to practitioners, students and house officers alike. Herein is compiled a wide coverage of this field of pediatrics in a small (1 lb.) book. The index and cross reference systems permit promptness and ease in finding desired material.

The basic structure of the book consists of a subdivision into seven chapters on the basis of anatomical systems. Symptoms and signs and their meaning are given first, followed by alphabetical listing of the diseases of the particular system with differential diagnosis and treatment. The last four chapters deal with laboratory procedures, nutrition, growth and development and drug prescriptions.

Non-essential material is not in this book; there isn't room for it. The encyclopedic nature of the contained data does not permit broadening out on any subject, and only the bare practical essentials are included. The authors present this work as a condensed "ready reminder" to be carried in a doctor's bag.

One finds unique and useful qualities in the completeness despite its small size, excellent cross reference system and up to date material, although the book is inadequate as a text, and equally inadequate as a reference book for detailed material about any problem. The author's preface should be read carefully, not only because therein is disclosed how best to use the manual, but also because the implication is emphasized that there is no substitute for brains, the only valid assumption upon which such a book can be logically and effectively used.

E. W. HOPKINS

The Epilepsies. Electro-Clinical Correlations. By HENRI GASTAUT. Translated by MARY A. B. BRAZIER, with a foreword by WILDER PENFIELD. 176 pp., $4.75. Charles C Thomas, Springfield, Ill.

This volume was written by one of France's most distinguished scientists, who is an acknowledged expert in the fields of electroencephalography and the epilepsies. It consists of seven chapters which deal in turn with the classification and clinical analysis of the epilepsies, the physiological interpretation of epileptic symptoms, the etiology, anatomical study, diagnosis, and treatment of epilepsies. In this slender monograph the author makes

no attempt at a comprehensive survey of the field, presenting instead a summary of his own research and clinical experiences at Marseilles, and this is its principle value. A certain amount of special information on the part of the reader is presumed, and for this reason the book will be of most interest to neurologists and neurosurgeons responsible for the treatment of epileptic patients. The discussions of etiology and therapy serve to emphasize the present confused state of knowledge of the mechanisms controlling the level of activity of nerve cells, and the need for continuing investigations of those controlling mechanisms.

V. B. MOUNTCASTLE

The Chemical Basis of Heredity. Edited by WILLIAM D. MCELROY AND BENTLEY GLASS. 848 pp., $12.50. The Johns Hopkins Press, Baltimore, Md.

This is the seventh in a series of symposia held at the McCollum-Pratt Institute. All have so far been distinguished by a high degree of excellence.

The report of this symposium is divided into seven sections. Section I deals with a discussion of cellular units as related to heredity. These include the role of the nucleus, the chromosome and the gene. There is further modification of the concept of the gene as the unit of genetic function. Thus the notion of the *muton*, *recon* and *cistron*, may well be a welcome addition to genetic concepts and genetic terminology. A good section is devoted to gene conversion, gene function and its relation to enzyme formation. The role of the cytoplasm in heredity is also presented.

Sections II, III and IV include mainly a discussion of the function of nucleic acids in protein synthesis and their role as transforming agents in bacteria, as shown in *Diplococcus pneumoniae* and *Hemophilus influenzae*.

Section IV represents a collection of papers on infectivity with *E. coli* phages, transduction and lysogeny. The nucleic acid moiety of reconstituted tobacco mosaic virus is reported to be unquestionably the determining genetic protein of the virus although the protein may possibly play some secondary role.

Section V is concerned exclusively with the composition and physical structure of the nucleic acid molecule. A cautious approach to the composition and model structure, coupled with bold concepts of the helical configuration of these compounds, provide exciting possibilities with promise of fruitful research in the ensuing years.

Section VI is devoted in part to the problem of the enzymatic synthesis of ribonucleotides and deoxyribonucleotides. This is one field that has made great strides during the last two years since the first demonstration that highly polymerized ribonucleotides were synthesized from 5′-nucleoside diphosphates by an enzyme from *Azotobacter vinelandii*. The other part includes a discussion of nucleic acid biosynthesis.

The final section, VII, includes a tempered appraisal of the topological difficulty in the replication of DNA as applied to the current Watson-Crick model. The possibility of a bipartite structure for transferrable phage DNA, was brought forth. One half may presumably be transferred to the progeny in big pieces and the other in small pieces. Thus, the process of recombination in phage which was not experimentally separable from replication may profitably be classified as fragmenting and non-fragmenting recombination.

As with the previous six symposia, the contributors were all leading workers in their respective fields of research. The volume concludes with a very lucid and cohesive summary.

No branch of science be it medicine, biology, microbiology, biochemistry or other related fields has been spared the impact of genetics in one form or another. To the medical student it is necessary for the elucidation and understanding of disease and to the student of basic sciences it is equally necessary to the elucidation and understanding of metabolic pathways.

In this sense and other ways, THE CHEMICAL BASIS OF HEREDITY should be a helpful and necessary addition to the bookshelf of all students concerned with the biological sciences.

VICTOR A. NAJJAR

Venous Return. By GERHARD A. BRECHER. 148 pp., $6.75. Grune & Stratton, Inc., New York, N. Y.

As Wiggers, with whom the author formerly worked, states in the foreword, "it is axiomatic that the heart can pump only as much blood as it receives!" Re-examination of the problem has been rendered feasible and productive by development of the electronic bristle flowmeter by Brecher and Praglin and by Scher, Weigert and Young. Brecher's monograph presents not only an excellent account of his own contributions but also a comprehensive treatise on the physiology of the venous side of the circulation. Individual chapter headings include the following: Historical considerations, Elementary hemodynamics, Factors affecting venous return, Methods for venous flow measurement, The respiratory pump, and several others. Effects of artificial respiration, lesions of the heart valves, and surgery on venous return are discussed. One is impressed with the wide ramifications of problems related to venous hemodynamics. The monograph is recommended whole-heartedly; it is a landmark in the literature of cardiovascular physiology.

VICTOR A. MCKUSICK

BOOKS RECEIVED FOR REVIEW

Annual Review of Medicine, Vol. 8, 1957. Edited by DAVID A. RYTAND. 530 pp., $7.00. Annual Reviews, Inc., Palo Alto, Cal.

Atlas of Clinical Endocrinology. Including Text of Diagnosis and Treatment. By H. LISSER AND ROBERTO F. ESCAMILLA. 476 pp., $18.75. The C. V. Mosby Co., St. Louis, Mo.

Calderwood's Orthopedic Nursing, 4th edition. Revised by CARROLL B. LARSON AND MARJORIE GOULD. 701 pp., $5.75. The C. V. Mosby Co., St. Louis, Missouri.

Clinical Pathology in General Practice. Laboratory Procedures and Interpretation. Specially Commissioned Articles from the British Medical Journal. 321 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

Die Periphere Innervation. By EUGEN LUDWIG. 210 pp., $5.00. Benno Schwabe & Co., Basel, Switzerland.

Essentials of Clinical Proctology, 3rd edition. By MANUEL G. SPIESMAN AND LOUIS MALOW. 316 pp., $8.75. Grune & Stratton, Inc., New York, N. Y.

Herz- und GefäßErkrankungen. Neue Wege einer funktionellen Differentialdiagnose. By R. VÖLKER. 166 pp., paper bound $7.77; cloth bound $8.25. Verlag Von Dr. Dietrich Steinkopff. Darmstadt.

Hutchison's Clinical Methods, 13th edition. By DONALD HUNTER AND R. R. BOMFORD. 452 pp., $6.00. J. B. Lippincott Co., Philadelphia, Pa.

Modern Therapy in Neurology. Edited by FRANCIS M. FORSTER with foreword by H. HOUSTON MERRITT. 792 pp., $12.00. The C. V. Mosby Co., St. Louis, Mo.

Perinatal Loss in Modern Obstetrics. By ROBERT E. L. NESBITT, JR. 432 pp., $12.50. F. A. Davis Co., Philadelphia, Pa.

Practical Gynecology, 2nd edition. By WALTER J. REICH AND MITCHELL J. NECHTOW. 648 pp., $12.50. J. B. Lippincott Co., Philadelphia, Pa.

Rypins' Medical Licensure Examinations, Topical Summaries and Questions, 8th edition.

Edited by WALTER L. BIERRING. 964 pp., $10.00. J. B. Lippincott Co., Philadelphia, Pa.

Signs and Symptoms, 3rd edition. Edited by CYRIL MITCHELL MACBRYDE. 973 pp., $12.00. J. B. Lippincott Co., Philadelphia, Pa.

Some Milestones in the History of Hematology. By CAMILLE DREYFUS. 87 pp., $4.50. Grune & Stratton, Inc., New York, N. Y.

A Textbook of Histology, 7th edition. By MAXIMOW BLOOM. 528 pp., $11.00. W. B. Saunders Co., Philadelphia, Pa.

Textbook of Pathology. By STANLEY L. ROBBINS. 1351 pp., $18.00. W. B. Saunders Co., Philadelphia, Pa.

Treatment of Burns. By CURTIS P. ARTZ AND ERIC REISS. 250 pp., $7.50. W. B. Saunders Co., Philadelphia, Pa.